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<p>(21) International Application Number: PCT/US94/00685 (22) International Filing Date: 12 January 1994 (12.01.94) (30) Priority Data: 08/003,303 12 January 1993 (12.01.93) US (60) Parent Application or Grant (63) Related by Continuation US 08/003,303 (CIP) Filed on 12 January 1993 (12.01.93) (71) Applicant (for all designated States except US): JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE [US/US]; 720 Rutland Avenue, Baltimore, MD 21205 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): LEE, Se-Jin [US/US]; 6711 Chokeberry Road, Baltimore, MD 21209 (US). (74) Agents: WETHERELL, John, R., Jr. et al.; Spensley Horn Jubas &amp; Lubitz, 5th floor, 1880 Century Park East, Los Angeles, CA 9067 (US).</p>		<p>(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published With international search report.</p>
<p>(54) Title: GROWTH DIFFERENTIATION FACTOR-9 (57) Abstract  Growth differentiation factor-9 (GDF-9) is disclosed along with its polynucleotide sequence and amino acid sequence. Also disclosed are diagnostic and therapeutic methods of using the GDF-9 polypeptide and polynucleotide sequences.</p>		

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## **GROWTH DIFFERENTIATION FACTOR-9**

This application is a continuation-in-part application of U.S. Serial No. 08/003,303, filed January 12, 1993.

### **BACKGROUND OF THE INVENTION**

#### **5        1.        *Field of the Invention***

The invention relates generally to growth factors and specifically to a new member of the transforming growth factor beta (TGF- $\beta$ ) superfamily, which is denoted, growth differentiation factor-9 (GDF-9).

#### **2.        *Description of Related Art***

10        The transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily encompasses a group of structurally-related proteins which affect a wide range of differentiation processes during embryonic development. The family includes, Mullerian inhibiting substance (MIS), which is required for normal male sex development (Behringer, *et al.*, *Nature*, 345:167, 1990), *Drosophila* decapentaplegic (DPP)  
15        gene product, which is required for dorsal-ventral axis formation and morphogenesis of the imaginal disks (Padgett, *et al.*, *Nature*, 325:81-84, 1987), the *Xenopus* Vg-1 gene product, which localizes to the vegetal pole of eggs ((Weeks, *et al.*, *Cell*, 51:861-867, 1987), the activins (Mason, *et al.*, *Biochem, Biophys. Res. Commun.*, 135:957-964, 1986), which can induce the formation  
20        of mesoderm and anterior structures in *Xenopus* embryos (Thomsen, *et al.*, *Cell*, 63:485, 1990), and the bone morphogenetic proteins (BMPs, osteogenin, OP-1) which can induce *de novo* cartilage and bone formation (Sampath, *et al.*, *J. Biol. Chem.*, 265:13198, 1990). The TGF- $\beta$ s can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis,

hematopoiesis, and epithelial cell differentiation (for review, see Massague, *Cell* 49:437, 1987).

The proteins of the TGF- $\beta$  family are initially synthesized as a large precursor protein which subsequently undergoes proteolytic cleavage at a cluster of basic residues approximately 110-140 amino acids from the C-terminus. The C-terminal regions of the proteins are all structurally related and the different family members can be classified into distinct subgroups based on the extent of their homology. Although the homologies within particular subgroups range from 70% to 90% amino acid sequence identity, the homologies between subgroups are significantly lower, generally ranging from only 20% to 50%. In each case, the active species appears to be a disulfide-linked dimer of C-terminal fragments. For most of the family members that have been studied, the homodimeric species has been found to be biologically active, but for other family members, like the inhibins (Ling, *et al.*, *Nature*, 321:779, 1986) and the TGF- $\beta$ s (Cheifetz, *et al.*, *Cell*, 48:409, 1987), heterodimers have also been detected, and these appear to have different biological properties than the respective homodimers.

The inhibins and activins were originally purified from follicular fluid and shown to have counteracting effects on the release of follicle-stimulating hormone by the pituitary gland. Although the mRNAs for all three inhibin/activin subunits ( $\alpha$ a,  $\beta$ A and  $\beta$ B) have been detected in the ovary, none of these appear to be ovary-specific (Meunier, *et al.*, *Proc.Natl.Acad.Sci. USA*, 85:247, 1988). MIS has also been shown to be expressed by granulosa cells and the effects of MIS on ovarian development have been documented both *in vivo* in transgenic mice expressing MIS ectopically (Behringer, *supra*) and *in vitro* in organ culture (Vigier, *et al.*, *Development*, 100:43, 1987).

Identification of new factors that are tissue-specific in their expression pattern will provide a greater understanding of that tissue's development and function.

## SUMMARY OF THE INVENTION

The present invention provides a cell growth and differentiation factor, GDF-9, a polynucleotide sequence which encodes the factor and antibodies which are immunoreactive with the factor. This factor appears to relate to various cell proliferative disorders, especially those involving ovarian tumors, such as granulosa cell tumors.

Thus, in one embodiment, the invention provides a method for detecting a cell proliferative disorder of ovarian origin and which is associated with GDF-9. In another embodiment, the invention provides a method of treating a cell proliferative disorder associated with abnormal levels of expression of GDF-9, by suppressing or enhancing GDF-9 activity.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows expression of GDF-9 mRNA in adult tissues.

FIGURE 2 shows nucleotide and predicted amino acid sequence of murine GDF-9. Consensus N-glycosylation signals are denoted by plain boxes. The putative tetrabasic processing sites are denoted by stippled boxes. The in-frame termination codons upstream of the putative initiating ATG and the consensus polyadenylation signals are underlined. The poly A tails are not shown. Numbers indicate nucleotide position relative to the 5' end.

FIGURE 3 shows the alignment of the C-terminal sequences of GDF-9 with other members of the TGF- $\beta$  family. The conserved cysteine residues are shaded. Dashes denote gaps introduced in order to maximize alignment.

FIGURE 4 shows amino acid homologies among the different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups.

FIGURE 5 shows the immunohistochemical localization of GDF-9 protein. Adjacent sections of an adult ovary were either stained with hematoxylin and eosin (FIGURE 5a) or incubated with immune (FIGURE 5b) or pre-immune (FIGURE 5c) serum at a dilution of 1:500. Anti-GDF-9 antiserum was prepared by expressing the C-terminal portion of murine GDF-9 (residues 308-441) in bacteria, excising GDF-9 protein from preparative SDS gels, and immunizing rabbits. Sites of antibody binding were visualized using the Vectastain ABC kit (Vector Labs).

FIGURE 6 shows a comparison of the predicted amino acid sequences of murine (top lines) and human (bottom lines) GDF-9. Numbers represent amino acid positions relative to the N-termini. Vertical lines represent sequence identities. Dots represent gaps introduced in order to maximize the alignment.

5 The clear box shows the predicted proteolytic processing sites. The shaded boxes show the cysteine residues in the mature region of the proteins. The bars at the bottom show a schematic of the pre-(clear) and mature (shaded) regions of GDF-9 with the percent sequence identities between the murine and human sequences shown below.

10 FIGURE 7 shows *in situ* hybridization to adult ovary sections using a GDF-9 RNA probe. [<sup>35</sup>S]-labeled anti-sense (FIGURE 7a and 7c) or sense (FIGURE 7 b and 7d) GDF-9 RNA probes were hybridized to adjacent paraffin-embedded sections of ovaries fixed in 4% paraformaldehyde. Sections were dipped in photographic emulsion, exposed, developed, and then stained with  
15 hematoxylin and eosin. Two representative fields are shown.

FIGURE 8 shows *in situ* hybridization to a postnatal day 4 ovary section using an antisense GDF-9 RNA probe. Sections were prepared as described for FIGURE 7. Following autoradiography and staining, the section was photographed under bright-field (FIGURE 8a) or dark-field (FIGURE 8b)  
20 illumination.

FIGURE 9 shows *in situ* hybridization to postnatal day 8 ovary sections using an antisense (FIGURE 9a) or sense (FIGURE 9b) GDF-9 RNA probe. Sections were prepared as described for FIGURE 7.

FIGURE 10 shows *in situ* hybridization to adult oviduct sections using an  
25 antisense (FIGURE 10a) or sense (FIGURE 10b) GDF-9 RNA probe. Sections were prepared as described for FIGURE 7.

FIGURE 11 shows *in situ* hybridization to an adult oviduct (0.5 days following fertilization) section using an antisense GDF-9 RNA probe. Sections were prepared as described for FIGURE 7. Following autoradiography and staining, the section was photographed under bright-field (FIGURE 11a) or dark-field (FIGURE 11b) illumination.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a growth and differentiation factor, GDF-9 and a polynucleotide sequence encoding GDF-9. Unlike other members of the TGF- $\beta$  superfamily, GDF-9 expression is highly tissue specific, being expressed in cells primarily in ovarian tissue. In one embodiment, the invention provides a method for detection of a cell proliferative disorder of the ovary, which is associated with GDF-9 expression. In another embodiment, the invention provides a method for treating a cell proliferative disorder associated with abnormal expression of GDF-9 by using an agent which suppresses or enhances GDF-9 activity.

The TGF- $\beta$  superfamily consists of multifunctional polypeptides that control proliferation, differentiation, and other functions in many cell types. Many of the peptides have regulatory, both positive and negative, effects on other peptide growth factors. The structural homology between the GDF-9 protein of this invention and the members of the TGF- $\beta$  family, indicates that GDF-9 is a new member of the family of growth and differentiation factors. Based on the known activities of many of the other members, it can be expected that GDF-9 will also possess biological activities that will make it useful as a diagnostic and therapeutic reagent.

For example, another regulatory protein that has been found to have structural homology with TGF- $\beta$  is inhibin, a specific and potent polypeptide inhibitor of the pituitary secretion of FSH. Inhibin has been isolated from ovarian follicular fluid. Because of its suppression of FSH, inhibin has potential to be used as a contraceptive in both males and females. GDF-9 may possess similar biological activity since it is also an ovarian specific peptide. Inhibin has also been shown to be useful as a marker for certain ovarian tumors (Lappohn, et al., *N. Engl. J. Med.*, 321:790, 1989). GDF-9 may also be useful as a marker

for identifying primary and metastatic neoplasms of ovarian origin. Similarly, GDF-9 may be useful as an indicator of developmental anomalies in prenatal screening procedures.

5 Another peptide of the TGF- $\beta$  family is MIS, produced by the testis and responsible for the regression of the Mullerian ducts in the male embryo. MIS has been shown to inhibit the growth of human ovarian cancer in nude mice (Donahoe, *et al.*, *Ann. Surg.*, 194:472, 1981). GDF-9 may function similarly and may, therefore, be useful as an anti-cancer agent, such as for the treatment of ovarian cancer.

10 GDF-9 may also function as a growth stimulatory factor and, therefore, be useful for the survival of various cell populations *in vitro*. In particular, if GDF-9 plays a role in oocyte maturation, it may be useful in *in vitro* fertilization procedures, e.g., in enhancing the success rate. Many of the members of the TGF- $\beta$  family are also important mediators of tissue repair. TGF- $\beta$  has been  
15 shown to have marked effects on the formation of collagen and causes a striking angiogenic response in the newborn mouse (Roberts, *et al.*, *Proc. Natl. Acad. Sci. USA*, 83:4167, 1986). GDF-9 may also have similar activities and may be useful in repair of tissue injury caused by trauma or burns for example.

20 The term "substantially pure" as used herein refers to GDF-9 which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify GDF-9 using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the GDF-9 polypeptide can also be determined by amino-terminal amino  
25 acid sequence analysis. GDF-9 polypeptide includes functional fragments of the polypeptide, as long as the activity of GDF-9 remains. Smaller peptides containing the biological activity of GDF-9 are included in the invention.

The invention provides polynucleotides encoding the GDF-9 protein. These polynucleotides include DNA, cDNA and RNA sequences which encode GDF-9. It is understood that all polynucleotides encoding all or a portion of GDF-9 are also included herein, as long as they encode a polypeptide with GDF-9 activity.

5 Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, GDF-9 polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for GDF-9 also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There

10 are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of GDF-9 polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specifically disclosed herein is a cDNA sequence for GDF-9 which is 1712 base

15 pairs in length and contains an open reading frame beginning with a methionine codon at nucleotide 29. The encoded polypeptide is 441 amino acids in length with a molecular weight of about 49.6 kD, as determined by nucleotide sequence analysis. The GDF-9 sequence contains a core of hydrophobic amino acids near the N-terminus, suggestive of a signal sequence

20 for secretion. GDF-9 contains four potential N-glycosylation sites at asparagine residues 163, 229, 258, and 325 and a putative tetrabasic proteolytic processing site (RRRR) at amino acids 303-306. The mature C-terminal fragment of GDF-9 is predicted to be 135 amino acids in length and have an unglycosylated molecular weight of about 15.6 kD, as determined by nucleotide

25 sequence analysis. One skilled in the art can modify, or partially or completely remove the glycosyl groups from the GDF-9 protein using standard techniques. Therefore, the functional protein or fragments thereof of the invention includes glycosylated, partially glycosylated and unglycosylated species of GDF-9.

The degree of sequence identity of GDF-9 with known TGF- $\beta$  family members ranges from a minimum of 21% with Mullerian inhibiting substance (MIS) to a maximum of 34% with bone morphogenetic protein-4 (BMP-4). GDF-9 specifically disclosed herein differs from the known family members in its pattern of cysteine residues in the C-terminal region. GDF-9 lacks the fourth cysteine of the seven cysteines present in other family members; in place of cysteine at this position, the GDF-9 sequence contains a serine residue. This GDF-9 does not contain a seventh cysteine residue elsewhere in the C-terminal region.

Minor modifications of the recombinant GDF-9 primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the GDF-9 polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of GDF-9 still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of a smaller active molecule which would have broader utility. For example, one can remove amino or carboxy terminal amino acids which are not required for GDF-9 biological activity.

The nucleotide sequence encoding the GDF-9 polypeptide of the invention includes the disclosed sequence and conservative variations thereof. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. The term

"conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

5 DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences and 2) antibody screening of expression libraries to detect cloned  
10 DNA fragments with shared structural features.

Preferably the GDF-9 polynucleotide of the invention is derived from a mammalian organism, and most preferably from a mouse, rat, or human. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate  
15 probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into  
20 account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from  
25 sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA

clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al.*, *Nucl. Acid Res.*, 9:879, 1981).

5 The development of specific DNA sequences encoding GDF-9 can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement  
10 of mRNA is eventually formed which is generally referred to as cDNA.

Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of  
15 introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not  
20 possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase  
25 chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded

DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, *et al.*, *Nucl. Acid Res.*, 11:2325, 1983).

- 5        A cDNA expression library, such as lambda gt11, can be screened indirectly for GDF-9 peptides having at least one epitope, using antibodies specific for GDF-9. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of GDF-9 cDNA.
- 10       DNA sequences encoding GDF-9 can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication.
- 15       However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In the present invention, the GDF-9 polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression

20       vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the GDF-9 genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well

25       as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg, *et al.*,

*Gene*, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein I, or polyhedrin promoters).

Polynucleotide sequences encoding GDF-9 can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention.

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the  $\text{CaCl}_2$  method using procedures well known in the art. Alternatively,  $\text{MgCl}_2$  or  $\text{RbCl}$  can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the GDF-9 of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect

or transform eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means  
5 including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

The invention includes antibodies immunoreactive with GDF-9 polypeptide or functional fragments thereof. Antibody which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct  
10 monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler, *et al.*, *Nature*, 256:495, 1975). The term antibody as used in this invention is meant to include intact molecules as well as fragments thereof, such as Fab and F(ab')<sub>2</sub>, which are capable of binding  
15 an epitopic determinant on GDF-9.

The term "cell-proliferative disorder" denotes malignant as well as non-malignant cell populations which often appear to differ from the surrounding tissue both morphologically and genotypically. The GDF-9 polynucleotide that is an antisense molecule is useful in treating malignancies of the various organ  
20 systems, particularly, for example, the ovaries. Essentially, any disorder which is etiologically linked to altered expression of GDF-9 could be considered susceptible to treatment with a GDF-9 suppressing reagent.

The invention provides a method for detecting a cell proliferative disorder of the ovary which comprises contacting an anti-GDF-9 antibody with a cell suspected  
25 of having a GDF-9 associated disorder and detecting binding to the antibody. The antibody reactive with GDF-9 is labeled with a compound which allows

detection of binding to GDF-9. For purposes of the invention, an antibody specific for GDF-9 polypeptide may be used to detect the level of GDF-9 in biological fluids and tissues. Any specimen containing a detectable amount of antigen can be used. A preferred sample of this invention is tissue of ovarian origin, specifically tissue containing granulosa cells or ovarian follicular fluid. The level of GDF-9 in the suspect cell can be compared with the level in a normal cell to determine whether the subject has a GDF-9-associated cell proliferative disorder. Preferably the subject is human.

The antibodies of the invention can be used in any subject in which it is desirable to administer *in vitro* or *in vivo* immunodiagnosis or immunotherapy. The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The antibodies of the invention can be bound to many different carriers and used to detect the presence of an antigen comprising the polypeptide of the invention. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled

in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

5 There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

10 Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific anti-  
15 hapten antibodies.

In using the monoclonal antibodies of the invention for the *in vivo* detection of antigen, the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to  
20 enable detection of the site having the antigen comprising a polypeptide of the invention for which the monoclonal antibodies are specific.

The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background. Further, it is desirable  
25 that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

As a rule, the dosage of detectably labeled monoclonal antibody for *in vivo* diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. Such dosages may vary, for example, depending on whether multiple injections are given, antigenic burden, and other factors known to those of skill in the art.

For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for *in vivo* diagnosis is that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for *in vivo* imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may readily be detected by conventional gamma cameras.

For *in vivo* diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are  $^{111}\text{In}$ ,  $^{97}\text{Ru}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{72}\text{As}$ ,  $^{89}\text{Zr}$ , and  $^{201}\text{Tl}$ .

The monoclonal antibodies of the invention can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and

paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include  $^{157}\text{Gd}$ ,  $^{55}\text{Mn}$ ,  $^{162}\text{Dy}$ ,  $^{52}\text{Cr}$ , and  $^{56}\text{Fe}$ .

The monoclonal antibodies of the invention can be used *in vitro* and *in vivo* to monitor the course of amelioration of a GDF-9-associated disease in a subject.

5 Thus, for example, by measuring the increase or decrease in the number of cells expressing antigen comprising a polypeptide of the invention or changes in the concentration of such antigen present in various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the GDF-9-associated disease is effective. The term "ameliorate"

10 denotes a lessening of the detrimental effect of the GDF-9-associated disease in the subject receiving therapy.

The present invention identifies a nucleotide sequence that can be expressed in an altered manner as compared to expression in a normal cell, therefore, it is possible to design appropriate therapeutic or diagnostic techniques directed

15 to this sequence. Thus, where a cell-proliferative disorder is associated with the expression of GDF-9, nucleic acid sequences that interfere with GDF-9 expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific GDF-9 mRNA, either by masking that mRNA with an antisense nucleic acid or

20 by cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense

25 nucleic acids interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely

to cause problems than larger molecules when introduced into the target GDF-9-producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal.Biochem.*, 172:289, 1988).

5     Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J.Amer.Med.*  
10    *Assn.*, 260:3030, 1988). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. *Tetrahymena*-type ribozymes  
15    recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a  
20    specific mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

The present invention also provides gene therapy for the treatment of cell proliferative disorders which are mediated by GDF-9 protein. Such therapy would achieve its therapeutic effect by introduction of the GDF-9 antisense  
25    polynucleotide into cells having the proliferative disorder. Delivery of antisense GDF-9 polynucleotide can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system.

Especially preferred for therapeutic delivery of antisense sequences is the use of targeted liposomes.

5 Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional  
10 retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a GDF-9 sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific.  
15 Retroviral vectors can be made target specific by inserting, for example, a polynucleotide encoding a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral  
20 genome to allow target specific delivery of the retroviral vector containing the GDF-9 antisense polynucleotide.

Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the  
25 structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines which have deletions of the packaging signal

include, but are not limited to  $\psi$ 2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes *gag*, *pol* and *env*, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for GDF-9 antisense polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0  $\mu$ m can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, *et al.*, *Trends Biochem. Sci.*, 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4)

accurate and effective expression of genetic information (Mannino, et al., *Biotechniques*, 6:682, 1988).

5 The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

10 Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

15 The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to  
20 distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and  
25 cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

Due to the expression of GDF-9 in the reproductive tract, there are a variety of applications using the polypeptide, polynucleotide and antibodies of the invention, related to contraception, fertility and pregnancy. GDF-9 could play a role in regulation of the menstrual cycle and, therefore, could be useful in various contraceptive regimens.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

**EXAMPLE 1**  
**IDENTIFICATION AND ISOLATION OF A NOVEL**  
**TGF- $\beta$  FAMILY MEMBER**

To identify a new member of the TGF- $\beta$  superfamily, degenerate oligonucleotides were designed which corresponded to two conserved regions among the known family members: one region spanning the two tryptophan residues conserved in all family members except MIS and the other region spanning the invariant cysteine residues near the C-terminus. These primers were used for polymerase chain reactions on mouse genomic DNA followed by subcloning the PCR products using restriction sites placed at the 5' ends of the primers, picking individual *E. coli* colonies carrying these subcloned inserts, and using a combination of random sequencing and hybridization analysis to eliminate known members of the superfamily.

GDF-9 was identified from a mixture of PCR products obtained with the primers SJL160 (5'-CCGGAATTCGGITGG(G/C/A)A(G/A/T/C)(G/C/A)A(G/A/T/C)TGG(A/G)TI(A/G)TI(T/G)CICC-3') (SEQUENCE ID NO. 1) and SJL153 (5'-CCGGAATTC(A/G)CAI(G/C)C(A/G)CAIC(T/C)(G/A/T/C)(C/G/T)TIG(T/C)I(G/A)(T/C)CAT-3') (SEQUENCE ID NO. 2). PCR using these primers was carried out with 2  $\mu$ g mouse genomic DNA at 94°C for 1 min, 50°C for 2 min, and 72°C for 2 min for 40 cycles.

PCR products of approximately 280 bp were gel-purified, digested with Eco RI, gel-purified again, and subcloned in the Bluescript vector (Stratagene, San Diego, CA). Bacterial colonies carrying individual subclones were picked into 96 well microtiter plates, and multiple replicas were prepared by plating the cells onto nitrocellulose. The replicate filters were hybridized to probes representing known members of the family, and DNA was prepared from non-hybridizing colonies for sequence analysis.

The primer combination of SJL160 and SJL153, yielded three known sequences (inhibin  $\beta$ B, BMP-2, and BMP-4) and one novel sequence (designated GDF-9) among 145 subclones analyzed.

5 RNA isolation and Northern analysis were carried out as described previously (Lee, S.J., *Mol. Endocrinol.* 4:1034, 1990). An oligo dT-primed cDNA library was prepared from 2.5-3  $\mu$ g of ovary poly A-selected RNA in the lambda ZAP II vector according to the instructions provided by Stratagene. The ovary library was not amplified prior to screening. Filters were hybridized as described previously (Lee, S.-J., *Proc. Natl. Acad. Sci. USA.*, 88:4250-4254, 10 1991). DNA sequencing of both strands was carried out using the dideoxy chain termination method (Sanger, *et al.*, *Proc. Natl. Acad. Sci., USA*, 74:5463-5467, 1977) and a combination of the S1 nuclease/exonuclease III strategy (Henikoff, S., *Gene*, 28:351-359, 1984) and synthetic oligonucleotide primers.

## EXAMPLE 2

### 15 EXPRESSION PATTERN AND SEQUENCE OF GDF-9

To determine the expression pattern of GDF-9, RNA samples prepared from a variety of adult tissues were screened by Northern analysis. Five micrograms of twice polyA-selected RNA prepared from each tissue were electrophoresed on formaldehyde gels, blotted and probed with GDF-9. As shown in Figure 1, 20 the GDF-9 probe detected a 1.7 kb mRNA expressed exclusively in the ovary.

A mouse ovary cDNA library of  $1.5 \times 10^6$  recombinant phage was constructed in lambda ZAP II and screened with a probe derived from the GDF-9 PCR product. The nucleotide sequence of the longest of nineteen hybridizing clones is shown in Figure 2. Consensus N-glycosylation signals are denoted 25 by plain boxes. The putative tetrabasic processing sites are denoted by

stippled boxes. The in-frame termination codons upstream of the putative initiating ATG and the consensus polyadenylation signals are underlined. The poly A tails are not shown. Numbers indicate nucleotide position relative to the 5' end. The 1712 bp sequence contains a long open reading frame beginning with a methionine codon at nucleotide 29 and potentially encoding a protein 441 amino acids in length with a molecular weight of 49.6 kD. Like other TGF- $\beta$  family members, the GDF-9 sequence contains a core of hydrophobic amino acids near the N-terminus suggestive of a signal sequence for secretion. GDF-9 contains four potential N-glycosylation sites at asparagine residues 163, 229, 258, and 325 and a putative tetrabasic proteolytic processing site (RRRR) at amino acids 303-306. The mature C-terminal fragment of GDF-9 is predicted to be 135 amino acids in length and have an unglycosylated molecular weight of 15.6 kD.

Although the C-terminal portion of GDF-9 clearly shows homology with the other family members, the sequence of GDF-9 is significantly diverged from those of the other family members (Figures 3 and 4). Figure 3 shows the alignment of the C-terminal sequences of GDF-9 with the corresponding regions of human GDF-1 (Lee, *Proc. Natl. Acad. Sci. USA*, 88:4250-4254, 1991), *Xenopus* Vg-1 (Weeks, et al., *Cell*, 51:861-867, 1987), human Vgr-1 (Celeste, et al., *Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human OP-1 (Ozkaynak, et al., *EMBO J.*, 9:2085-2093, 1990), human BMP-5 (Celeste, et al., *Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), *Drosophila* 60A (Wharton, et al., *Proc. Natl. Acad. Sci. USA*, 88:9214-9218, 1991), human BMP-2 and 4 (Wozney, et al., *Science*, 242:1528-1534, 1988), *Drosophila* DPP (Padgett, et al., *Nature*, 325:81-84, 1987), human BMP-3 (Wozney, et al., *Science*, 242:1528-1534, 1988), human MIS (Cate, et al., *Cell*, 45:685-698, 1986), human inhibin ,  $\beta$ A, and  $\beta$ B (Mason, et al., *Biochem, Biophys. Res. Commun.*, 135:957-964, 1986), human TGF- $\beta$ 1 (Derynck, et al., *Nature*, 316:701-705, 1985), human TGF- $\beta$ 2 (deMartin, et al., *EMBO J.*, 6:3673-3677, 1987), human TGF- $\beta$ 3

(ten Dijke, *et al.*, *Proc. Natl. Acad. Sci. USA*, 85:4715-4719, 1988), chicken TGF- $\beta$ 4 (Jakowlew, *et al.*, *Mol. Endocrinol.*, 2:1186-1195, 1988), and *Xenopus* TGF- $\beta$ 5 (Kondaiah, *et al.*, *J. Biol. Chem.*, 265:1089-1093, 1990). The conserved cysteine residues are shaded. Dashes denote gaps introduced in order to maximize the alignment.

Figure 4 shows the amino acid homologies among the different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups.

The degree of sequence identify with known family members ranges from a minimum of 21% with MIS to a maximum of 34% with BMP-4. Hence, GDF-9 is comparable to MIS in its degree of sequence divergence from the other members of this superfamily. Moreover, GDF-9 shows no significant sequence homology to other family members in the pro-region of the molecule. GDF-9 also differs from the known family members in its pattern of cysteine residues in the C-terminal region. GDF-9 lacks the fourth cysteine of the seven cysteines that are present in all other family members; in place of cysteine at this position, the GDF-9 sequence contains a serine residue. In addition, GDF-9 does not contain a seventh cysteine residue elsewhere in the C-terminal region.

**EXAMPLE 3**  
**IMMUNOCHEMICAL LOCALIZATION OF GDF-9**  
**IN THE ZONA PELLUCIDA**

To determine whether GDF-9 mRNA was translated, sections of adult ovaries  
5 were incubated with antibodies directed against recombinant GDF-9 protein.  
In order to raise antibodies against GDF-9, portions of GDF-9 cDNA spanning  
amino acids 30 to 295 (pro-region) or 308 to 441 (mature region) were cloned  
into the T7-based pET3 expression vector (provided by F.W. Studier,  
Brookhaven National Laboratory), and the resulting plasmids were transformed  
10 into the BL21 (DE3) bacterial strain. Total cell extracts from isopropyl  $\beta$ -D-  
thiogalactoside-induced cells were electrophoresed on SDS/polyacrylamide  
gels, and the GDF-9 protein fragments were excised, mixed with Freund's  
adjuvant, and used to immunize rabbits by standard methods known to those  
of skill in the art. All immunizations were carried out by Spring Valley Lab  
15 (Sykesville, MD). The presence of GDF-9-reactive antibodies in the sera of  
these rabbits was assessed by Western analysis of bacterially-expressed  
protein fragments. The resulting serum was shown to react with the bacterially-  
expressed protein by Western analysis.

For immunohistochemical studies, ovaries were removed from adult mice, fixed  
20 in 4% paraformaldehyde, embedded in paraffin, and sectioned. Sites of  
antibody binding were detected by using the Vectastain ABC kit, according to  
the instructions provided by Vector Laboratories. FIGURE 5 shows the  
immunohistochemical localization of GDF-9 protein. Adjacent sections of an  
adult ovary were either stained with hematoxylin and eosin (FIGURE 5a) or  
25 incubated with immune (FIGURE 5b) or pre-immune (FIGURE 5c) serum at a  
dilution of 1:500. As shown in FIGURE 5b, the antiserum detected protein

solely in oocytes. No staining was detected using pre-immune serum (FIGURE 5c). Hence, GDF-9 protein appears to translated *in vivo* by oocytes.

#### **EXAMPLE 4**

#### **ISOLATION OF HUMAN GDF-9**

5 In order to isolate a cDNA clone encoding human GDF-9, a cDNA library was constructed in lambda ZAP II using poly A-selected RNA prepared from an adult human ovary. From this library, a cDNA clone containing the entire human GDF-9 coding sequence was identified using standard screening techniques as in Example 1 and using the murine GDF-9 clone as a probe.

10 A comparison of the predicted amino acid sequences of murine (top lines) and human (bottom lines) GDF-9 is shown in FIGURE 6. Numbers represent amino acid positions relative to the N-termini. Vertical lines represent sequence identities. Dots represent gaps introduced in order to maximize the alignment. The clear box shows the predicted proteolytic processing sites. The shaded

15 boxes show the cysteine residues in the mature region of the proteins. The bars at the bottom show a schematic of the pre-(clear) and mature (shaded) regions of GDF-9 with the percent sequence identities between the murine and human sequences shown below.

20 Like murine GDF-9, human GDF-9 contains a hydrophobic leader sequence, a putative RXXR proteolytic cleavage site, and a C-terminal region containing the hallmarks of other TGF- $\beta$  family members. Murine and human GDF-9 are 64% identical in the pro- region and 90% identical in the predicted mature region of the molecule. The high degree of homology between the two sequences suggests that human GDF-9 plays an important role during

25 embryonic development and/or in the adult ovary.

### **EXAMPLE 5**

#### **NUCLEIC ACID DETECTION OF EXPRESSION OF GDF-9 IN OOCYTES**

In order to localize the expression of GDF-9 in the ovary, *in situ* hybridization to mouse ovary sections was carried out using an antisense GDF-9 RNA probe. FIGURE 7 shows *in situ* hybridization to adult ovary sections using a GDF-9 RNA probe. [<sup>35</sup>S]-labeled anti-sense (FIGURE 7a and 7c) or sense (FIGURE 7 b and 7d) GDF-9 RNA probes were hybridized to adjacent paraffin-embedded sections of ovaries fixed in 4% paraformaldehyde. Sections were dipped in photographic emulsion, exposed, developed, and then stained with hematoxylin and eosin. Two representative fields are shown.

As shown in FIGURES 7a and 7c, GDF-9 mRNA was detected primarily in oocytes in adult ovaries. Every oocyte (regardless of the stage of follicular development) examined showed GDF-9 expression, and no expression was detected in any other cell types. No hybridization was seen using a control GDF-9 sense RNA probe (FIGURE 7b and 7d). Hence, GDF-9 expression appears to be oocyte-specific in adult ovaries.

To determine the pattern of expression of GDF-9 mRNA during ovarian development, sections of neonatal ovaries were probed with a GDF-9 RNA probe. FIGURE 8 shows *in situ* hybridization to a postnatal day 4 ovary section using an antisense GDF-9 RNA probe. Sections were prepared as described for FIGURE 7. Following autoradiography and staining, the section was photographed under bright-field (FIGURE 8a) or dark-field (FIGURE 8b) illumination.

FIGURE 9 shows *in situ* hybridization to postnatal day 8 ovary sections using an antisense (FIGURE 9a) or sense (FIGURE 9b) GDF-9 RNA probe. Sections were prepared as described for FIGURE 7.

GDF-9 mRNA expression was first detected at the onset of follicular development. This was most clearly evident at postnatal day 4, where only oocytes that were present in follicles showed GDF-9 expression (FIGURE 8); no expression was seen in oocytes that were not surrounded by granulosa cells. By postnatal day 8, every oocyte appeared to have undergone follicular development, and every oocyte showed GDF-9 expression (FIGURE 9).

To determine whether GDF-9 was also expressed following ovulation, sections of mouse oviducts were examined by *in situ* hybridization. FIGURE 10 shows *in situ* hybridization to adult oviduct sections using an antisense (FIGURE 10a) or sense (FIGURE 10b) GDF-9 RNA probe. Sections were prepared as described for FIGURE 7.

FIGURE 11 shows *in situ* hybridization to an adult oviduct (0.5 days following fertilization) section using an antisense GDF-9 RNA probe. Sections were prepared as described for FIGURE 7. Following autoradiography and staining, the section was photographed under bright-field (FIGURE 11a) or dark-field (FIGURE 11b) illumination.

As shown in FIGURE 10, GDF-9 was expressed by oocytes that had been released into the oviduct. However, the expression of GDF-9 mRNA turned off rapidly following fertilization of the oocytes; by day 0.5 following fertilization, only some embryos (such as the one shown in FIGURE 11) expressed GDF-9 mRNA, and by day 1.5, all embryos were negative for GDF-9 expression.

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

## **SUMMARY OF SEQUENCES**

Sequence ID No. 1 is the nucleotide sequence for the primer, SJL160, for GDF-9 (page 24, lines 15 and 16);

5      Sequence ID No. 2 is the nucleotide sequence for the primer, SJL153, for GDF-9 (page 24, lines 17 and 18);

Sequence ID No. 3 is the nucleotide and deduced amino acid sequence for GDF-9 (Figure 2);

Sequence ID No. 4 is the deduced amino acid sequence for GDF-9 (Figure 2);

10      Sequence ID No. 5 is the amino acid sequence of the C-terminus of GDF-3 (Figure 3);

Sequence ID No. 6 is the amino acid sequence of the C-terminus of GDF-9 (Figure 3);

Sequence ID No. 7 is the amino acid sequence of the C-terminus of GDF-1 (Figure 3);

15      Sequence ID No. 8 is the amino acid sequence of the C-terminus of Vg-1 (Figure 3);

Sequence ID No. 9 is the amino acid sequence of the C-terminus of Vgr-1 (Figure 3);

20      Sequence ID No. 10 is the amino acid sequence of the C-terminus of OP-1 (Figure 3);

Sequence ID No. 11 is the amino acid sequence of the C-terminus of BMP-5 (Figure 3);

Sequence ID No. 12 is the amino acid sequence of the C-terminus of 60A (Figure 3);

5      Sequence ID No. 13 is the amino acid sequence of the C-terminus of BMP-2 (Figure 3);

Sequence ID No. 14 is the amino acid sequence of the C-terminus of BMP-4 (Figure 3);

10      Sequence ID No. 15 is the amino acid sequence of the C-terminus of DPP (Figure 3);

Sequence ID No. 16 is the amino acid sequence of the C-terminus of BMP-3 (Figure 3);

Sequence ID No. 17 is the amino acid sequence of the C-terminus of MIS (Figure 3);

15      Sequence ID No. 18 is the amino acid sequence of the C-terminus of inhibin  $\alpha$  (Figure 3);

Sequence ID No. 19 is the amino acid sequence of the C-terminus of inhibin  $\beta$ A (Figure 3);

20      Sequence ID No. 20 is the amino acid sequence of the C-terminus of inhibin  $\beta$ B (Figure 3);

Sequence ID No. 21 is the amino acid sequence of the C-terminus of TGF- $\beta$ 1 (Figure 3);

Sequence ID No. 22 is the amino acid sequence of the C-terminus of TGF- $\beta$ 2 (Figure 3);

5      Sequence ID No. 23 is the amino acid sequence of the C-terminus of TGF- $\beta$ 3 (Figure 3);

Sequence ID No. 24 is the amino acid sequence of the C-terminus of TGF- $\beta$ 4 (Figure 3);

10      Sequence ID No. 25 is the amino acid sequence of the C-terminus of TGF- $\beta$ 5 (Figure 3); and

Sequence ID No. 26 is the amino acid sequence of human GDF-9 (Figure 6).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE JOHNS HOPKINS UNIVERSITY

(ii) TITLE OF INVENTION: GROWTH DIFFERENTIATION FACTOR-9

5 (iii) NUMBER OF SEQUENCES: 26

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Spensley Horn Jubas & Lubitz  
(B) STREET: 1880 Century Park East, Suite 500  
10 (C) CITY: Los Angeles  
(D) STATE: California  
(E) COUNTRY: US  
(F) ZIP: 90067

(v) COMPUTER READABLE FORM:

15 (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

20 (A) APPLICATION NUMBER:  
(B) FILING DATE: 12-JAN-1994  
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

25 (A) NAME: Wetherell, Jr. Ph.D., John R.  
(B) REGISTRATION NUMBER: 31,678  
(C) REFERENCE/DOCKET NUMBER: FD3288

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (619) 455-5100  
(B) TELEFAX: (619) 455-5110

30 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 base pairs  
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

5 (B) CLONE: SJL160

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..35

10 (D) OTHER INFORMATION: /note= "Where "B" occurs, B =  
inosine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGGAATTCG GBTGGVANVA NTGGRTBRTB KCBCC

35

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 33 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20 (vii) IMMEDIATE SOURCE:

(B) CLONE: SJL153

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..33

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCGGAATTCT CADSCRCADC YNBTDGYDRY CAT

33

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1712 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: GDF-9

(ix) FEATURE:

10

- (A) NAME/KEY: CDS
- (B) LOCATION: 29..1351

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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20	TTG GGG GTT TGC TGC TTT GCC TGG CTG TGT TTT CTT AGT AGC CTT AGC Leu Gly Val Cys Cys Phe Ala Trp Leu Cys Phe Leu Ser Ser Leu Ser 10 15 20	100
25	TCT CAG GCT TCT ACT GAA GAA TCC CAG AGT GGA GCC AGT GAA AAT GTG Ser Gln Ala Ser Thr Glu Glu Ser Gln Ser Gly Ala Ser Glu Asn Val 25 30 35 40	148
30	GAG TCT GAG GCA GAC CCC TGG TCC TTG CTG CTG CCT GTA GAT GGG ACT Glu Ser Glu Ala Asp Pro Trp Ser Leu Leu Leu Pro Val Asp Gly Thr 45 50 55	196
35	GAC AGG TCT GGC CTC TTG CCC CCC CTC TTT AAG GTT CTA TCT GAT AGG Asp Arg Ser Gly Leu Leu Pro Pro Leu Phe Lys Val Leu Ser Asp Arg 60 65 70	244
40	CGA GGT GAG ACC CCT AAG CTG CAG CCT GAC TCC AGA GCA CTC TAC TAC Arg Gly Glu Thr Pro Lys Leu Gln Pro Asp Ser Arg Ala Leu Tyr Tyr 75 80 85	292

	ATG AAA AAG CTC TAT AAG ACG TAT GCT ACC AAA GAG GGG GTT CCC AAA	340
	Met Lys Lys Leu Tyr Lys Thr Tyr Ala Thr Lys Glu Gly Val Pro Lys	
	90 95 100	
5	CCC AGC AGA AGT CAC CTC TAC AAT ACC GTC CGG CTC TTC AGT CCC TGT	388
	Pro Ser Arg Ser His Leu Tyr Asn Thr Val Arg Leu Phe Ser Pro Cys	
	105 110 115 120	
	GCC CAG CAA GAG CAG GCA CCC AGC AAC CAG GTG ACA GGA CCG CTG CCG	436
	Ala Gln Gln Glu Gln Ala Pro Ser Asn Gln Val Thr Gly Pro Leu Pro	
	125 130 135	
10	ATG GTG GAC CTG CTG TTT AAC CTG GAC CGG GTG ACT GCC ATG GAA CAC	484
	Met Val Asp Leu Leu Phe Asn Leu Asp Arg Val Thr Ala Met Glu His	
	140 145 150	
15	TTG CTC AAA TCG GTC TTG CTA TAC ACT CTG AAC AAC TCT GCC TCT TCC	532
	Leu Leu Lys Ser Val Leu Leu Tyr Thr Leu Asn Asn Ser Ala Ser Ser	
	155 160 165	
	TCC TCC ACT GTG ACC TGT ATG TGT GAC CTT GTG GTA AAG GAG GCC ATG	580
	Ser Ser Thr Val Thr Cys Met Cys Asp Leu Val Val Lys Glu Ala Met	
	170 175 180	
20	TCT TCT GGC AGG GCA CCC CCA AGA GCA CCG TAC TCA TTC ACC CTG AAG	628
	Ser Ser Gly Arg Ala Pro Pro Arg Ala Pro Tyr Ser Phe Thr Leu Lys	
	185 190 195 200	
	AAA CAC AGA TGG ATT GAG ATT GAT GTG ACC TCC CTC CTT CAG CCC CTA	676
	Lys His Arg Trp Ile Glu Ile Asp Val Thr Ser Leu Leu Gln Pro Leu	
	205 210 215	
25	GTG ACC TCC AGC GAG AGG AGC ATT CAC CTG TCT GTC AAT TTT ACA TGC	724
	Val Thr Ser Ser Glu Arg Ser Ile His Leu Ser Val Asn Phe Thr Cys	
	220 225 230	
30	ACA AAA GAC CAG GTG CCA GAG GAC GGA GTG TTT AGC ATG CCT CTC TCA	772
	Thr Lys Asp Gln Val Pro Glu Asp Gly Val Phe Ser Met Pro Leu Ser	
	235 240 245	
	GTG CCT CCT TCC CTC ATC TTG TAT CTC AAC GAC ACA AGC ACC CAG GCC	820
	Val Pro Pro Ser Leu Ile Leu Tyr Leu Asn Asp Thr Ser Thr Gln Ala	
	250 255 260	

	TAC CAC TCT TGG CAG TCT CTT CAG TCC ACC TGG AGG CCT TTA CAG CAT	868
	Tyr His Ser Trp Gln Ser Leu Gln Ser Thr Trp Arg Pro Leu Gln His	
	265 270 275 280	
5	CCC GGC CAG GCC GGT GTG GCT GCC CGT CCC GTG AAA GAG GAA GCT ACT	916
	Pro Gly Gln Ala Gly Val Ala Ala Arg Pro Val Lys Glu Glu Ala Thr	
	285 290 295	
	GAG GTG GAA AGA TCT CCC CGG CGC CGT CGA GGG CAG AAA GCC ATC CGC	964
	Glu Val Glu Arg Ser Pro Arg Arg Arg Arg Gly Gln Lys Ala Ile Arg	
	300 305 310	
10	TCC GAA GCG AAG GGG CCA CTT CTT ACA GCA TCC TTC AAC CTC AGC GAA	1012
	Ser Glu Ala Lys Gly Pro Leu Leu Thr Ala Ser Phe Asn Leu Ser Glu	
	315 320 325	
	TAC TTC AAA CAG TTT CTT TTC CCC CAA AAC GAG TGT GAA CTC CAT GAC	1060
15	Tyr Phe Lys Gln Phe Leu Phe Pro Gln Asn Glu Cys Glu Leu His Asp	
	330 335 340	
	TTC AGA CTG AGT TTT AGT CAG CTC AAA TGG GAC AAC TGG ATC GTG GCC	1108
	Phe Arg Leu Ser Phe Ser Gln Leu Lys Trp Asp Asn Trp Ile Val Ala	
	345 350 355 360	
20	CCG CAC AGG TAC AAC CCT AGG TAC TGT AAA GGG GAC TGT CCT AGG GCG	1156
	Pro His Arg Tyr Asn Pro Arg Tyr Cys Lys Gly Asp Cys Pro Arg Ala	
	365 370 375	
	GTC AGG CAT CGG TAT GGC TCT CCT GTG CAC ACC ATG GTC CAG AAT ATA	1204
	Val Arg His Arg Tyr Gly Ser Pro Val His Thr Met Val Gln Asn Ile	
	380 385 390	
25	ATC TAT GAG AAG CTG GAC CCT TCA GTG CCA AGG CCT TCG TGT GTG CCG	1252
	Ile Tyr Glu Lys Leu Asp Pro Ser Val Pro Arg Pro Ser Cys Val Pro	
	395 400 405	
	GGC AAG TAC AGC CCC CTG AGT GTG TTG ACC ATT GAA CCC GAC GGC TCC	1300
30	Gly Lys Tyr Ser Pro Leu Ser Val Leu Thr Ile Glu Pro Asp Gly Ser	
	410 415 420	
	ATC GCT TAC AAA GAG TAC GAA GAC ATG ATA GCT ACG AGG TGC ACC TGT	1348
	Ile Ala Tyr Lys Glu Tyr Glu Asp Met Ile Ala Thr Arg Cys Thr Cys	
	425 430 435 440	

CGT TAGCATGGGG GCCACTTCAA CAAGCCTGCC TGGCAGAGCA ATGCTGTGGG 1401  
 Arg

CCTTAGAGTG CCTGGGCAGA GAGCTTCCTG TGACCAGTCT CTCCGTGCTG CTCAGTGCAC 1461

5 ACTGTGTGAG CGGGGGAAGT GTGTGTGTGT GGATGAGCAC ATCGAGTGCA GTGTCCGTAG 1521

GTGTAAAGGG CAACTCACT GGTCGTTGCC ATAAACCAAG TGAAATGTAA CTCATTGGA 1581

GAGCTCTTTC TCCCCACGAG TGTAGTTTTC AGTGGACAGA TTTGTTAGCA TAAGTCTCGA 1641

GTAGAATGTA GCTGTGAACA TGTCAGAGTG CTGTGGTTTT ATGTGACGGA AGAATAAACT 1701

GTTGATGGCA T 1712

10 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 441 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Leu Pro Ser Asn Phe Leu Leu Gly Val Cys Cys Phe Ala Trp  
 1 5 10 15

Leu Cys Phe Leu Ser Ser Leu Ser Ser Gln Ala Ser Thr Glu Glu Ser  
 20 20 25 30

Gln Ser Gly Ala Ser Glu Asn Val Glu Ser Glu Ala Asp Pro Trp Ser  
 35 40 45

Leu Leu Leu Pro Val Asp Gly Thr Asp Arg Ser Gly Leu Leu Pro Pro  
 50 55 60

Leu Phe Lys Val Leu Ser Asp Arg Arg Gly Glu Thr Pro Lys Leu Gln  
 25 65 70 75 80

Pro Asp Ser Arg Ala Leu Tyr Tyr Met Lys Lys Leu Tyr Lys Thr Tyr  
 85 90 95

	Ala Thr Lys Glu Gly Val Pro Lys Pro Ser Arg Ser His Leu Tyr Asn	
	100	110
	Thr Val Arg Leu Phe Ser Pro Cys Ala Gln Gln Glu Gln Ala Pro Ser	
	115	125
5	Asn Gln Val Thr Gly Pro Leu Pro Met Val Asp Leu Leu Phe Asn Leu	
	130	140
	Asp Arg Val Thr Ala Met Glu His Leu Leu Lys Ser Val Leu Leu Tyr	
	145	160
10	Thr Leu Asn Asn Ser Ala Ser Ser Ser Ser Thr Val Thr Cys Met Cys	
	165	175
	Asp Leu Val Val Lys Glu Ala Met Ser Ser Gly Arg Ala Pro Pro Arg	
	180	190
	Ala Pro Tyr Ser Phe Thr Leu Lys Lys His Arg Trp Ile Glu Ile Asp	
	195	205
15	Val Thr Ser Leu Leu Gln Pro Leu Val Thr Ser Ser Glu Arg Ser Ile	
	210	220
	His Leu Ser Val Asn Phe Thr Cys Thr Lys Asp Gln Val Pro Glu Asp	
	225	240
20	Gly Val Phe Ser Met Pro Leu Ser Val Pro Pro Ser Leu Ile Leu Tyr	
	245	255
	Leu Asn Asp Thr Ser Thr Gln Ala Tyr His Ser Trp Gln Ser Leu Gln	
	260	270
	Ser Thr Trp Arg Pro Leu Gln His Pro Gly Gln Ala Gly Val Ala Ala	
	275	285
25	Arg Pro Val Lys Glu Glu Ala Thr Glu Val Glu Arg Ser Pro Arg Arg	
	290	300
	Arg Arg Gly Gln Lys Ala Ile Arg Ser Glu Ala Lys Gly Pro Leu Leu	
	305	320
30	Thr Ala Ser Phe Asn Leu Ser Glu Tyr Phe Lys Gln Phe Leu Phe Pro	
	325	335

Gln Asn Glu Cys Glu Leu His Asp Phe Arg Leu Ser Phe Ser Gln Leu  
340 345 350

Lys Trp Asp Asn Trp Ile Val Ala Pro His Arg Tyr Asn Pro Arg Tyr  
355 360 365

5 Cys Lys Gly Asp Cys Pro Arg Ala Val Arg His Arg Tyr Gly Ser Pro  
370 375 380

Val His Thr Met Val Gln Asn Ile Ile Tyr Glu Lys Leu Asp Pro Ser  
385 390 395 400

10 Val Pro Arg Pro Ser Cys Val Pro Gly Lys Tyr Ser Pro Leu Ser Val  
405 410 415

Leu Thr Ile Glu Pro Asp Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp  
420 425 430

Met Ile Ala Thr Arg Cys Thr Cys Arg  
435 440

15 (2) INFORMATION FOR SEQ ID NO:5:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 117 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: GDF-3

(ix) FEATURE:

25 (A) NAME/KEY: Protein

(B) LOCATION: 1..117

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Lys Arg Arg Ala Ala Ile Ser Val Pro Lys Gly Phe Cys Arg Asn Phe  
1 5 10 15

5

10

Phe Met Gln Ala Leu Met His Met Ala Asp Pro Lys Val Pro Lys Ala  
65                      70                      75                      80

Ser Asp Lys Asn Val Ile Leu Arg His Tyr Glu Asp Met Val Val Asp  
100 105 110

15

(i) SEQUENCE CHARACTERISTICS:

20

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: GDF-9

(ix) **FEATURE:**

25

(A) NAME/KEY: Protein

(B) LOCATION: 1..118

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Phe Asn Leu Ser Glu Tyr Phe Lys Gln Phe Leu Phe Pro Gln Asn Glu  
1 5 10 15

Cys Glu Leu His Asp Phe Arg Leu Ser Phe Ser Gln Leu Lys Trp Asp  
20 25 30

Asn Trp Ile Val Ala Pro His Arg Tyr Asn Pro Arg Tyr Cys Lys Gly  
35 40 45

5            Asp Cys Pro Arg Ala Val Arg His Arg Tyr Gly Ser Pro Val His Thr  
             50                            55                            60

Met	Val	Gln	Asn	Ile	Ile	Tyr	Glu	Lys	Leu	Asp	Pro	Ser	Val	Pro	Arg
65					70					75					80

Pro Ser Cys Val Pro Gly Lys Tyr Ser Pro Leu Ser Val Leu Thr Ile  
10 85 90 95

Glu Pro Asp Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp Met Ile Ala  
100 105 110

Thr Arg Cys Thr Cys Arg  
115

15 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 122 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: GDF-1

(ix) **FEATURE:**

(A) NAME/KEY: Protein

(B) LOCATION: 1..122

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly Ala  
1                   5                   10                   15

Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp His  
                     20                    25                    30  
 Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly  
                     35                    40                    45  
 5 Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala  
                     50                    55                    60  
 Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro Gly  
                     65                    70                    75                    80  
 10 Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile Ser  
                     85                    90                    95  
 Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr Glu  
                     100                    105                    110  
 Asp Met Val Val Asp Glu Cys Gly Cys Arg  
                     115                    120

15 (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 118 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: Vg-1

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..118

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Arg Arg Lys Arg Ser Tyr Ser Lys Leu Pro Phe Thr Ala Ser Asn Ile  
   1                    5                    10                    15

Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys Asp Val Gly Trp Gln  
20 25 30

Asn Trp Val Ile Ala Pro Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly  
35 40 45

5 Glu Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly Ser Asn His Ala  
50 55 60

Ile Leu Gln Thr Leu Val His Ser Ile Glu Pro Glu Asp Ile Pro Leu  
65 70 75 80

10 Pro Cys Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met Leu Phe Tyr  
85 90 95

Asp Asn Asn Asp Asn Val Val Leu Arg His Tyr Glu Asn Met Ala Val  
100 105 110

Asp Glu Cys Gly Cys Arg  
115

15 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 118 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
(B) CLONE: Vgr-1

25 (ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..118

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Arg Val Ser Ser Ala Ser Asp Tyr Asn Ser Ser Glu Leu Lys Thr Ala  
1 5 10 15

Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly  
35 40 45

5            Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala  
               50                          55                          60

Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys  
65                      70                      75                      80

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe  
10 85 90 95

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val  
100 105 110

Arg Ala Cys Gly Cys His  
115

15 (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 118 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: OP-1

(ix) FEATURE:

25 (A) NAME/KEY: Protein

(B) LOCATION: 1..118

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser Asp Gln Arg Gln Ala  
1 5 10 15

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly  
35 40 45

5            Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala Thr Asn His Ala  
               50                      55                      60

Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Glu Thr Val Pro Lys  
65                      70                      75                      80

Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val Leu Tyr Phe  
10 85 90 95

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val  
100 105 110

Arg Ala Cys Gly Cys His  
115

15 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 118 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

20 (D) TOPOLOGY: linear

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: BMP-5

(ix) FEATURE:

25 (A) NAME/KEY: Protein

(B) LOCATION: 1..118

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Arg Met Ser Ser Val Gly Asp Tyr Asn Thr Ser Glu Gln Lys Gln Ala  
1 5 10 15

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln  
20 25 30

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly  
35 40 45

5            Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala  
               50                          55                          60

Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys  
65                      70                      75                      80

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe  
10 85 90 95

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val  
100 105 110

Arg Ser Cys Gly Cys His  
115

15 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 118 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: 60A

(ix) FEATURE:

25 (A) NAME/KEY: Protein

(B) LOCATION: 1..118

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg Ser  
1 5 10 15

	Cys	Gln	Met	Gln	Thr	Leu	Tyr	Ile	Asp	Phe	Lys	Asp	Leu	Gly	Trp	His	
						20			25					30			
	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Gly	Ala	Phe	Tyr	Cys	Ser	Gly	
			35					40					45				
5	Glu	Cys	Asn	Phe	Pro	Leu	Asn	Ala	His	Met	Asn	Ala	Thr	Asn	His	Ala	
		50					55					60					
	Ile	Val	Gln	Thr	Leu	Val	His	Leu	Leu	Glu	Pro	Lys	Lys	Val	Pro	Lys	
	65					70					75				80		
	Pro	Cys	Cys	Ala	Pro	Thr	Arg	Leu	Gly	Ala	Leu	Pro	Val	Leu	Tyr	His	
10					85					90					95		
	Leu	Asn	Asp	Glu	Asn	Val	Asn	Leu	Lys	Lys	Tyr	Arg	Asn	Met	Ile	Val	
				100					105					110			
	Lys	Ser	Cys	Gly	Cys	His											
				115													

15 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 117 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: BMP-2

(ix) FEATURE:

25 (A) NAME/KEY: Protein

(B) LOCATION: 1..117

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Glu	Lys	Arg	Gln	Ala	Lys	His	Lys	Gln	Arg	Lys	Arg	Leu	Lys	Ser	Ser
1				5				10					15		

Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly  
35 40 45

5            Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala  
               50                          55                          60

Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala  
65                      70                      75                      80

10 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp  
85 90 95

Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu  
100 105 110

Gly Cys Gly Cys Arg  
115

15 (2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 117 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: BMP-4

(ix) FEATURE:

25 (A) NAME/KEY: Protein

(B) LOCATION: 1..117

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn  
1 5 10 15

	Cys	Arg	Arg	His	Ser	Leu	Tyr	Val	Asp	Phe	Ser	Asp	Val	Gly	Trp	Asn	
									20					25		30	
	Asp	Trp	Ile	Val	Ala	Pro	Pro	Gly	Tyr	Gln	Ala	Phe	Tyr	Cys	His	Gly	
									35					40		45	
5	Asp	Cys	Pro	Phe	Pro	Leu	Ala	Asp	His	Leu	Asn	Ser	Thr	Asn	His	Ala	
														50	55	60	
	Ile	Val	Gln	Thr	Leu	Val	Asn	Ser	Val	Asn	Ser	Ser	Ile	Pro	Lys	Ala	
														65	70	75	80
	Cys	Cys	Val	Pro	Thr	Glu	Leu	Ser	Ala	Ile	Ser	Met	Leu	Tyr	Leu	Asp	
10														85	90	95	
	Glu	Tyr	Asp	Lys	Val	Val	Leu	Lys	Asn	Tyr	Gln	Glu	Met	Val	Val	Glu	
														100	105	110	
	Gly	Cys	Gly	Cys	Arg												

15 (2) INFORMATION FOR SEQ ID NO:15:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 118 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: DPP

(ix) FEATURE:

25 (A) NAME/KEY: Protein

(B) LOCATION: 1..118

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Lys	Arg	His	Ala	Arg	Arg	Pro	Thr	Arg	Arg	Lys	Asn	His	Asp	Asp	Thr
1						5				10					15

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asp  
20 25 30

Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly  
35 40 45

5 Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser Thr Asn His Ala  
50 55 60

Val Val Gln Thr Leu Val Asn Asn Met Asn Pro Gly Lys Val Pro Lys  
65 70 75 80

10 Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met Leu Tyr Leu  
85 90 95

Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr Gln Glu Met Thr Val  
100 105 110

Val Gly Cys Gly Cys Arg  
115

15 (2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
(B) CLONE: BMP-3

(ix) FEATURE:  
25 (A) NAME/KEY: Protein  
(B) LOCATION: 1..119

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gln Thr Leu Lys Lys Ala Arg Arg Lys Gln Trp Ile Glu Pro Arg Asn  
1 5 10 15

	Cys	Ala	Arg	Arg	Tyr	Leu	Lys	Val	Asp	Phe	Ala	Asp	Ile	Gly	Trp	Ser	
				20					25					30			
	Glu	Trp	Ile	Ile	Ser	Pro	Lys	Ser	Phe	Asp	Ala	Tyr	Tyr	Cys	Ser	Gly	
			35					40					45				
5	Ala	Cys	Gln	Phe	Pro	Met	Pro	Lys	Ser	Leu	Lys	Pro	Ser	Asn	His	Ala	
		50					55					60					
	Thr	Ile	Gln	Ser	Ile	Val	Arg	Ala	Val	Gly	Val	Val	Pro	Gly	Ile	Pro	
	65					70					75					80	
	Glu	Pro	Cys	Cys	Val	Pro	Glu	Lys	Met	Ser	Ser	Leu	Ser	Ile	Leu	Phe	
10					85					90					95		
	Phe	Asp	Glu	Asn	Lys	Asn	Val	Val	Leu	Lys	Val	Tyr	Pro	Asn	Met	Thr	
					100				105					110			
	Val	Glu	Ser	Cys	Ala	Cys	Arg										
						115											

15 (2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 115 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: MIS

(ix) FEATURE:

25 (A) NAME/KEY: Protein

(B) LOCATION: 1..115

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Pro	Gly	Arg	Ala	Gln	Arg	Ser	Ala	Gly	Ala	Thr	Ala	Ala	Asp	Gly	Pro
1				5				10					15		

Leu Ile Pro Glu Thr Tyr Gln Ala Asn Asn Cys Gln Gly Val Cys Gly  
35 40 45

5 Trp Pro Gln Ser Asp Arg Asn Pro Arg Tyr Gly Asn His Val Val Leu  
50 55 60

Leu Leu Lys Met Gln Ala Arg Gly Ala Ala Leu Ala Arg Pro Pro Cys  
65 70 75 80

10 Cys Val Pro Thr Ala Tyr Ala Gly Lys Leu Leu Ile Ser Leu Ser Glu 85 90 95

Glu Arg Ile Ser Ala His His Val Pro Asn Met Val Ala Thr Glu Cys  
100 105 110

Gly Cys Arg  
115

15 (2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 121 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: Inhibin alpha

(ix) FEATURE:

25 (A) NAME/KEY: Protein

(B) LOCATION: 1..121

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Leu Arg Leu Leu Gln Arg Pro Pro Glu Glu Pro Ala Ala His Ala Asn  
1 5 10 15

	Cys	His	Arg	Val	Ala	Leu	Asn	Ile	Ser	Phe	Gln	Glu	Leu	Gly	Trp	Glu	
				20					25					30			
	Arg	Trp	Ile	Val	Tyr	Pro	Pro	Ser	Phe	Ile	Phe	His	Tyr	Cys	His	Gly	
			35					40					45				
5	Gly	Cys	Gly	Leu	His	Ile	Pro	Pro	Asn	Leu	Ser	Leu	Pro	Val	Pro	Gly	
		50					55					60					
	Ala	Pro	Pro	Thr	Pro	Ala	Gln	Pro	Tyr	Ser	Leu	Leu	Pro	Gly	Ala	Gln	
	65					70					75					80	
	Pro	Cys	Cys	Ala	Ala	Leu	Pro	Gly	Thr	Met	Arg	Pro	Leu	His	Val	Arg	
10					85					90					95		
	Thr	Thr	Ser	Asp	Gly	Gly	Tyr	Ser	Phe	Lys	Tyr	Glu	Thr	Val	Pro	Asn	
				100					105					110			
	Leu	Leu	Thr	Gln	His	Cys	Ala	Cys	Ile								
			115					120									

15 (2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- |    |                             |
|----|-----------------------------|
|    | (A) LENGTH: 121 amino acids |
|    | (B) TYPE: amino acid        |
| 20 | (C) STRANDEDNESS: single    |
|    | (D) TOPOLOGY: linear        |

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: Inhibin betaA

(ix) FEATURE:

- |    |                       |
|----|-----------------------|
| 25 | (A) NAME/KEY: Protein |
|    | (B) LOCATION: 1..121  |

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Arg	Arg	Arg	Arg	Arg	Gly	Leu	Glu	Cys	Asp	Gly	Lys	Val	Asn	Ile	Cys
1				5					10					15	

	Cys	Lys	Lys	Gln	Phe	Phe	Val	Ser	Phe	Lys	Asp	Ile	Gly	Trp	Asn	Asp	
				20					25						30		
	Trp	Ile	Ile	Ala	Pro	Ser	Gly	Tyr	His	Ala	Asn	Tyr	Cys	Glu	Gly	Glu	
		35						40					45				
5	Cys	Pro	Ser	His	Ile	Ala	Gly	Thr	Ser	Gly	Ser	Ser	Leu	Ser	Phe	His	
		50					55					60					
	Ser	Thr	Val	Ile	Asn	His	Tyr	Arg	Met	Arg	Gly	His	Ser	Pro	Phe	Ala	
	65					70					75					80	
	Asn	Leu	Lys	Ser	Cys	Cys	Val	Pro	Thr	Lys	Leu	Arg	Pro	Met	Ser	Met	
10					85					90					95		
	Leu	Tyr	Tyr	Asp	Asp	Gly	Gln	Asn	Ile	Ile	Lys	Lys	Asp	Ile	Gln	Asn	
				100					105						110		
	Met	Ile	Val	Glu	Glu	Cys	Gly	Cys	Ser								
				115				120									

15 (2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- |    |                             |
|----|-----------------------------|
|    | (A) LENGTH: 120 amino acids |
|    | (B) TYPE: amino acid        |
| 20 | (C) STRANDEDNESS: single    |
|    | (D) TOPOLOGY: linear        |

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: Inhibin betaB

(ix) FEATURE:

- |    |                       |
|----|-----------------------|
| 25 | (A) NAME/KEY: Protein |
|    | (B) LOCATION: 1..120  |

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Arg	Ile	Arg	Lys	Arg	Gly	Leu	Glu	Cys	Asp	Gly	Arg	Thr	Asn	Leu	Cys
1				5					10					15	

Trp Ile Ile Ala Pro Thr Gly Tyr Tyr Gly Asn Tyr Cys Glu Gly Ser  
35 40 45

5 Cys Pro Ala Tyr Leu Ala Gly Val Pro Gly Ser Ala Ser Ser Phe His  
50 55 60

Thr Ala Val Val Asn Gln Tyr Arg Met Arg Gly Leu Asn Pro Gly Thr  
65 70 75 80

Val Asn Ser Cys Cys Ile Pro Thr Lys Leu Ser Thr Met Ser Met Leu  
10 85 90 95

Tyr Phe Asp Asp Glu Tyr Asn Ile Val Lys Arg Asp Val Pro Asn Met  
100 105 110

Ile Val Glu Glu Cys Gly Cys Ala  
115 120

15 (2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 114 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: TGF-beta1

(ix) FEATURE:

25 (A) NAME/KEY: Protein

(B) LOCATION: 1..114

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Arg Arg Ala Leu Asp Thr Asn Tyr Cys Phe Ser Ser Thr Glu Lys Asn  
1 5 10 15

Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys Asp Leu Gly Trp  
 20 25 30  
 Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala Asn Phe Cys Leu Gly  
 35 40 45  
 5 Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr Ser Lys Val Leu  
 50 55 60  
 Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala Ala Pro Cys Cys  
 65 70 75 80  
 10 Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr Tyr Val Gly Arg  
 85 90 95  
 Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val Arg Ser Cys Lys  
 100 105 110  
 Cys Ser

15 (2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 114 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: TGF-beta2

25 (ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..114

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Lys Arg Ala Leu Asp Ala Ala Tyr Cys Phe Arg Asn Val Gln Asp Asn  
 1 5 10 15

Lys Trp Ile His Glu Pro Lys Gly Tyr Asn Ala Asn Phe Cys Ala Gly  
35 40 45

5 Ala Cys Pro Tyr Leu Trp Ser Ser Asp Thr Gln His Ser Arg Val Leu  
50 55 60

Ser Leu Tyr Asn Thr Ile Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys  
65 70 75 80

Val Ser Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Ile Gly Lys  
10 85 90 95

Thr Pro Lys Ile Glu Gln Leu Ser Asn Met Ile Val Lys Ser Cys Lys  
100 105 110

15 (2) INFORMATION FOR SEQ ID NO:23:

(A) LENGTH: 114 amino acids

20 (D) TOPOLOGY: linear

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: TGF-beta3

(ix) FEATURE:

25 (A) NAME/KEY: Protein

(B) LOCATION: 1..114

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Arg Ala Leu Asp Thr Asn Tyr Cys Phe Arg Asn Leu Glu Glu Asn  
1                   5                   10                   15

	Cys	Cys	Val	Arg	Pro	Leu	Tyr	Ile	Asp	Phe	Arg	Gln	Asp	Leu	Gly	Trp	
				20					25					30			
	Lys	Trp	Val	His	Glu	Pro	Lys	Gly	Tyr	Tyr	Ala	Asn	Phe	Cys	Ser	Gly	
			35					40					45				
5	Pro	Cys	Pro	Tyr	Leu	Arg	Ser	Ala	Asp	Thr	Thr	His	Ser	Thr	Val	Leu	
		50					55					60					
	Gly	Leu	Tyr	Asn	Thr	Leu	Asn	Pro	Glu	Ala	Ser	Ala	Ser	Pro	Cys	Cys	
	65					70					75				80		
	Val	Pro	Gln	Asp	Leu	Glu	Pro	Leu	Thr	Ile	Leu	Tyr	Tyr	Val	Gly	Arg	
10				85						90					95		
	Thr	Pro	Lys	Val	Glu	Gln	Leu	Ser	Asn	Met	Val	Val	Lys	Ser	Cys	Lys	
				100					105					110			
	Cys	Ser															

15 (2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 116 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

(B) CLONE: TGF-beta4

(ix) FEATURE:

(A) NAME/KEY: Protein

25 (B) LOCATION: 1..116

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Arg	Arg	Asp	Leu	Asp	Thr	Asp	Tyr	Cys	Phe	Gly	Pro	Gly	Thr	Asp	Glu
1				5					10					15	

```

Lys Asn Cys Cys Val Arg Pro Leu Tyr Ile Asp Phe Arg Lys Asp Leu
      20                      25                      30

Gln Trp Lys Trp Ile His Glu Pro Lys Gly Tyr Met Ala Asn Phe Cys
      35                      40                      45

5  Met Gly Pro Cys Pro Tyr Ile Trp Ser Ala Asp Thr Gln Tyr Thr Lys
      50                      55                      60

Val Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala Ala Pro
65                      70                      75                      80

10 Cys Cys Val Pro Gln Thr Leu Asp Pro Leu Pro Ile Ile Tyr Tyr Val
      85                      90                      95

Gly Arg Asn Val Arg Val Glu Gln Leu Ser Asn Met Val Val Arg Ala
      100                     105                     110

Cys Lys Cys Ser
      115

```

15 (2) INFORMATION FOR SEQ ID NO:25:

```

      (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 114 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
20      (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

```

(vii) IMMEDIATE SOURCE:
      (B) CLONE: TGF-beta5

```

```

25      (ix) FEATURE:
            (A) NAME/KEY: Protein
            (B) LOCATION: 1..114

```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```

Lys Arg Gly Val Gly Gln Glu Tyr Cys Phe Gly Asn Asn Gly Pro Asn
1      5                      10                      15

```

Cys Cys Val Lys Pro Leu Tyr Ile Asn Phe Arg Lys Asp Leu Gly Trp  
                     20                    25                    30  
 Lys Trp Ile His Glu Pro Lys Gly Tyr Glu Ala Asn Tyr Cys Leu Gly  
                     35                    40                    45  
 5 Asn Cys Pro Tyr Ile Trp Ser Met Asp Thr Gln Tyr Ser Lys Val Leu  
                     50                    55                    60  
 Ser Leu Tyr Asn Gln Asn Asn Pro Gly Ala Ser Ile Ser Pro Cys Cys  
                     65                    70                    75                    80  
 10 Val Pro Asp Val Leu Glu Pro Leu Pro Ile Ile Tyr Tyr Val Gly Arg  
                     85                    90                    95  
 Thr Ala Lys Val Glu Gln Leu Ser Asn Met Val Val Arg Ser Cys Asn  
                     100                    105                    110  
 Cys Ser

15 (2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:  
     (A) LENGTH: 454 amino acids  
     (B) TYPE: amino acid  
     (C) STRANDEDNESS: single  
 20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:  
     (B) CLONE: HUMAN GDF-9

25 (ix) FEATURE:  
     (A) NAME/KEY: Protein  
     (B) LOCATION: 1..454

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

	Met	Ala	Arg	Pro	Asn	Lys	Phe	Leu	Leu	Trp	Phe	Cys	Cys	Phe	Ala	Trp	
	1				5					10					15		
	Leu	Cys	Phe	Pro	Ile	Ser	Leu	Gly	Ser	Gln	Ala	Ser	Gly	Gly	Glu	Ala	
				20					25					30			
5	Gln	Ile	Ala	Ala	Ser	Ala	Glu	Leu	Glu	Ser	Gly	Ala	Met	Pro	Trp	Ser	
			35					40					45				
	Leu	Leu	Gln	His	Ile	Asp	Glu	Arg	Asp	Arg	Ala	Gly	Leu	Leu	Pro	Ala	
		50				55						60					
10	Leu	Phe	Lys	Val	Leu	Ser	Val	Gly	Arg	Gly	Gly	Ser	Pro	Arg	Leu	Gln	
	65					70					75					80	
	Pro	Asp	Ser	Arg	Ala	Leu	His	Tyr	Met	Lys	Lys	Leu	Tyr	Lys	Thr	Tyr	
					85					90					95		
	Ala	Thr	Lys	Glu	Gly	Ile	Pro	Lys	Ser	Asn	Arg	Ser	His	Leu	Tyr	Asn	
				100					105					110			
15	Thr	Val	Arg	Leu	Phe	Thr	Pro	Cys	Thr	Arg	His	Lys	Gln	Ala	Pro	Gly	
			115					120					125				
	Asp	Gln	Val	Thr	Gly	Ile	Leu	Pro	Ser	Val	Glu	Leu	Leu	Phe	Asn	Leu	
		130					135					140					
20	Asp	Arg	Ile	Thr	Thr	Val	Glu	His	Leu	Leu	Lys	Ser	Val	Leu	Leu	Tyr	
	145					150					155					160	
	Asn	Ile	Asn	Asn	Ser	Val	Ser	Phe	Ser	Ser	Ala	Val	Lys	Cys	Val	Cys	
				165						170				175			
	Asn	Leu	Met	Ile	Lys	Glu	Pro	Lys	Ser	Ser	Ser	Arg	Thr	Leu	Gly	Arg	
			180						185					190			
25	Ala	Pro	Tyr	Ser	Phe	Thr	Phe	Asn	Ser	Gln	Phe	Glu	Phe	Gly	Lys	Lys	
			195					200					205				
	His	Lys	Trp	Ile	Gln	Ile	Asp	Val	Thr	Ser	Leu	Leu	Gln	Pro	Leu	Val	
		210					215						220				
30	Ala	Ser	Asn	Lys	Arg	Ser	Ile	His	Met	Ser	Ile	Asn	Phe	Thr	Cys	Met	
	225					230					235					240	

	Lys Asp Gln Leu Glu His Pro Ser Ala Gln Asn Gly Leu Phe Asn Met	
	245	250 255
	Thr Leu Val Ser Pro Ser Leu Ile Leu Tyr Leu Asn Asp Thr Ser Ala	
	260	265 270
5	Gln Ala Tyr His Ser Trp Tyr Ser Leu His Tyr Lys Arg Arg Pro Ser	
	275	280 285
	Gln Gly Pro Asp Gln Glu Arg Ser Leu Ser Ala Tyr Pro Val Gly Glu	
	290	295 300
10	Glu Ala Ala Glu Asp Gly Arg Ser Ser His His Arg His Arg Arg Gly	
	305	310 315 320
	Gln Glu Thr Val Ser Ser Glu Leu Lys Lys Pro Leu Gly Pro Ala Ser	
	325	330 335
	Phe Asn Leu Ser Glu Tyr Phe Arg Gln Phe Leu Leu Pro Gln Asn Glu	
	340	345 350
15	Cys Glu Leu His Asp Phe Arg Leu Ser Phe Ser Gln Leu Lys Trp Asp	
	355	360 365
	Asn Trp Ile Val Ala Pro His Arg Tyr Asn Pro Arg Tyr Cys Lys Gly	
	370	375 380
20	Asp Cys Pro Arg Ala Val Gly His Arg Tyr Gly Ser Pro Val His Thr	
	385	390 395 400
	Met Val Gln Asn Ile Ile Tyr Glu Lys Leu Asp Ser Ser Val Pro Arg	
	405	410 415
	Pro Ser Cys Val Pro Ala Lys Tyr Ser Pro Leu Ser Val Leu Thr Ile	
	420	425 430
25	Glu Pro Asp Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp Met Ile Ala	
	435	440 445
	Thr Lys Cys Thr Cys Arg	
	450	

## **CLAIMS**

1. Substantially pure growth differentiation factor-9 (GDF-9) and functional fragments thereof.
2. An isolated polynucleotide sequence encoding the GDF-9 polypeptide of claim 1.
3. The polynucleotide sequence of claim 2, wherein the polynucleotide is isolated from a mammalian cell.
4. The polynucleotide of claim 3, wherein the mammalian cell is selected from the group consisting of mouse, rat, and human cell.
5. An expression vector including the polynucleotide of claim 2.
6. The vector of claim 5, wherein the vector is a plasmid.
7. The vector of claim 5, wherein the vector is a virus.
8. A host cell stably transformed with the vector of claim 5.
9. The host cell of claim 8, wherein the cell is prokaryotic.
10. The host cell of claim 8, wherein the cell is eukaryotic.
11. Antibodies reactive with the polypeptide of claim 1 or fragments thereof.
12. The antibodies of claim 11, wherein the antibodies are polyclonal.

13. The antibodies of claim 11, wherein the antibodies are monoclonal.
14. A method of detecting a cell proliferative disorder comprising contacting the antibody of claim 11 with a specimen of a subject suspected of having a GDF-9 associated disorder and detecting binding of the antibody.
15. The method of claim 14, wherein the cell proliferative disorder is an ovarian tumor.
16. The method of claim 14, wherein the detecting is *in vivo*.
17. The method of claim 16, wherein the antibody is detectably labeled.
18. The method of claim 17, wherein the detectable label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound and a chemiluminescent compound.
19. The method of claim 14, wherein the detection is *in vitro*.
20. The method of claim 19, wherein the antibody is detectably labeled.
21. The method of claim 20, wherein the label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemoluminescent compound and an enzyme.
22. A method of treating a cell proliferative disorder associated with expression of GDF-9, comprising contacting the cells with a reagent which suppresses the GDF-9 activity.

23. The method of claim 22, wherein the reagent is an anti-GDF-9 antibody.
24. The method of claim 22, wherein the reagent is a GDF-9 antisense sequence.
25. The method of claim 22, wherein the cell proliferative disorder is an ovarian tumor.
26. The method of claim 22, wherein the reagent which suppresses GDF-9 activity is introduced to a cell using a vector.
27. The method of claim 26, wherein the vector is a colloidal dispersion system.
28. The method of claim 27, wherein the colloidal dispersion system is a liposome.
29. The method of claim 28, wherein the liposome is essentially target specific.
30. The method of claim 29, wherein the liposome is anatomically targeted.
31. The method of claim 29, wherein the liposome is mechanistically targeted.
32. The method of claim 31, wherein the mechanistic targeting is passive.
33. The method of claim 31, wherein the mechanistic targeting is active.

34. The method of claim 33, wherein the liposome is actively targeted by coupling with a moiety selected from the group consisting of a sugar, a glycolipid, and a protein.
35. The method of claim 34, wherein the protein moiety is an antibody.
36. The method of claim 35, wherein the vector is a virus.
37. The method of claim 36, wherein the virus is an RNA virus.
38. The method of claim 37, wherein the RNA virus is a retrovirus.
39. The method of claim 38, wherein the retrovirus is essentially target specific.

1/15

- 1.7 kb

ADRENAL  
PANCREAS  
INTESTINE  
SPLEEN  
KIDNEY  
LUNG  
HEART  
BRAIN  
OVARY  
LIVER  
THYMUS  
UTERUS  
SEMINAL VESICLE  
TESTIS  
10.5 d PLACENTA

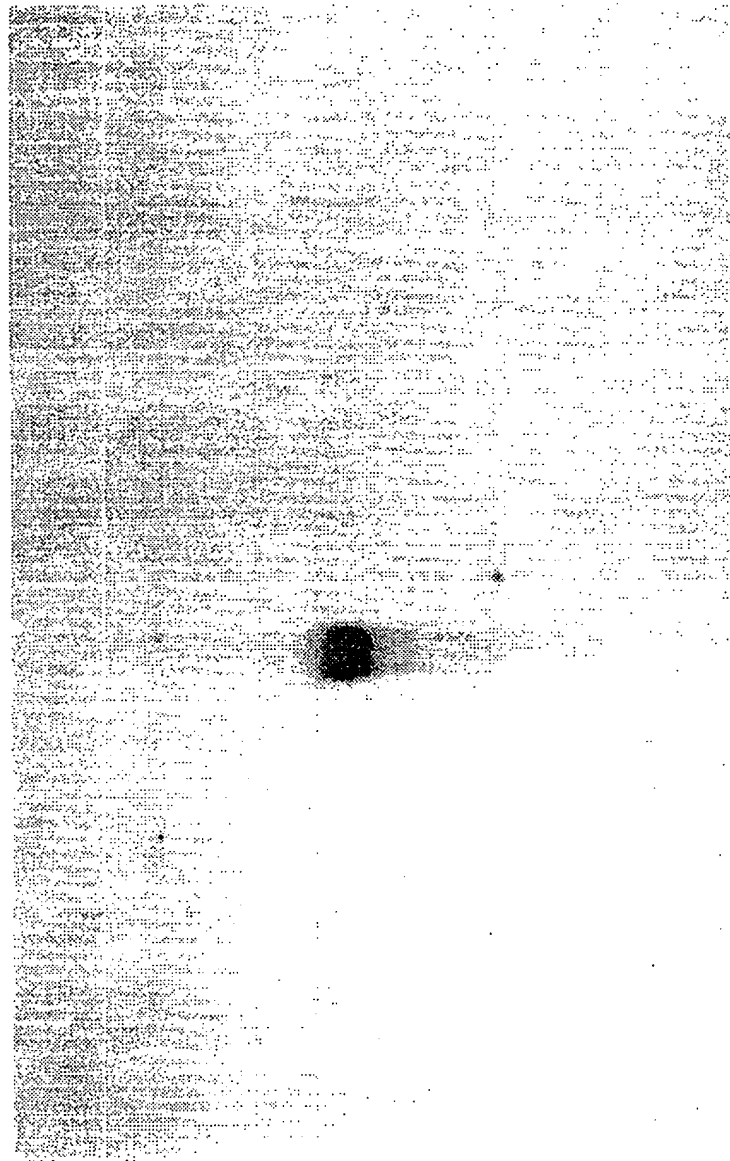


FIG. 1

SUBSTITUTE SHEET

1 ATGCGTTCCTCTTAGTTTCTTCCAAAGTCATGGCACTTCCAGCAACTTCCTGTGTGGGGT 60  
M A L P S N F L L G V  
61 TTGCTGCTTTGCCCTGGCTGTGTTTCTTAGTAGCCTTAGCTCTCAGGCTTCTACTGAAGA 120  
C C F A W L C F L S S L S S Q A S T E E  
121 ATCCCAGAGTGGAGCCAGTGAAATGTGGAGTCTGAGGCAGACCCCTGGTCTCTGCTGCT 180  
S Q S G A S E N V E S E A D P W S L L L  
181 GCCTGTAGATGGGACTGACAGGTCTGGCCTCTTGCCCCCCTCTTTAAAGTTCTATCTGA 240  
P V D G T D R S G L L P P L F K V L S D  
241 TAGGCGAGGTGAGACCCCTAAGCTGCAGCCTGACTCCAGAGCACTCTACTACATGAAAAA 300  
R R G E T P K L Q P D S R A L Y Y M K K  
301 GCTCTATAAGACGTATGCTACCAAGAGGGGTTCCTCCAAACCCAGCAGAGTCACCTCTA 360  
L Y K T Y A T K E G V P K P S R S H L Y  
361 CAATACCGTCCGGCTCTTCAGTCCCTGTGCCAGCAAGAGCAGGCCACCCAGCAACCAGGT 420  
N T V R L F S P C A Q Q E Q A P S N Q V  
421 GACAGGACCGCTGCCGATGGTGGACCTGCTGTTTAACCTGGACCGGTGACTGCCATGGA 480  
T G P L P M V D L L F N L D R V T A M E  
481 ACACTTGCTCAAAATCGGTCTTGCTATACACTCTGAACAACCTGCTCTCTCTCCTCCAC 540  
H L L K S V L L Y T L N N S A S S S T  
541 TGTGACCTGTATGTGACCTTGTGTAAGGAGGCCATGTCTTCTGTGGCAGGCAACCCC 600  
V T C M C D L V V K E A M S S G R A P P

2/15

FIG.2a

601 AAGAGCACCGTACTCATTCACCCCTGAAGAAACACAGATGGATTGAGATTGATGTGACCTC 660  
 R A P Y S F T L K K H R W I E I D V T S  
 661 CCTCCTCAGCCCCCTAGTGACCTCCAGCGAGAGGAGCATTCACCTGTCTGTCAATTTTAC 720  
 L L Q P L V T S S E R S I H L S V N F T  
 721 ATGCACAAAGACCAAGGTGCCAGAGGACGGAGTGTTTAGCATGCCCTCTCTCAGTGCCCTCC 780  
 C T K D Q V P E D G V F S M P L S V P P  
 781 TTCCCTCATCTTGTAATCTCAACGACACAAAGCACCCAGGCTACCACTCTTGGCAGTCTCT 840  
 S L I L Y L N D T S T Q A Y H S W Q S L  
 841 TCAGTCCACCTGGAGGCCCTTTACAGCATCCCGGCCAGGCCGGTGTGGCTGCCCGTCCCGT 900  
 Q S T W R P L Q H P G Q A G V A A R P V  
 901 GAAAGAGGAGCTACTGAGGTGGAAGATCTCCCGGCCGCTCAGGGCAGAAAGCCAT 960  
 K E E A T E V E R S P R R R R G Q K A I  
 961 CCGTCCGAGCGAAGGGCCACTTCTTACAGCATCCTTCAACCTCAGCGAATACTTCAA 1020  
 R S E A K G P L L T A S F N L S E Y F K  
 1021 ACAGTTTCTTTTCCCCCAAACGAGTGTGAACCTCCATGACTTCAGACTGAGTTTGTAGTCA 1080  
 Q F L F P Q N E C E L H D F R L S F S Q  
 1081 GCTCAATGGGACAACTGGATCGTGGCCCCCGCACAGGTACAACCCCTAGGTACTGTAAAGG 1140  
 L K W D N W I V A P H R Y N P R Y C K G

1141 G G A C T G T C C T A G G G C G G T C A G G C A T C G G T A T G G C T C T C C T G T G C A C A C C A T G G T C C A G A A 1200  
D C P R A V R H R Y G S P V H T M V Q N  
1201 T A T A A T C T A T G A G A A G C T G G A C C C T T C A G T G C C A A G G C C T T C G T G T G T G C C G G C A A G T A 1260  
I I Y E K L D P S V P R P S C V P G K Y  
1261 C A G C C C C C T G A G T G T G T G A C C A T T G A A C C C G A C G G C T C C A T C G C T T A C A A A G A G T A C G A 1320  
S P L S V L T I E P D G S I A Y K E Y E  
1321 A G A C A T G A T A G C T A C G A G G T G C A C C T G T C G T T A G C A T G G G G C C A C T T C A A C A A G C C T G C 1380  
D M I A T R C T C R \*  
1381 C T G G C A G A G C A A T G C T G T G G G C C T T A G A G T G C C T G G G C A G A G A G C T T C C T G T G A C C A G T C 1440  
1441 T C T C C G T G C T C A G T G C A C A C T G T G T G A G C G G G G A A G T G T G T G T G T G G A T G A G C A 1500  
1501 C A T C G A G T G C A G T G T C C G T A G G T G T A A G G G C A C A C T C A C T G G T C G T T G C C A T A A A C C A A 1560  
1561 G T G A A A T G T A A C T C A T T T G G A G A G C T C T T T C T C C C C A C G A G T G T A G T T T T C A G T G G A C A G 1620  
1621 A T T T G T T A G C A T A A G T C T C G A G T A G A A T G T A G C T G T G A A C A T G T C A G A G T G C T G T G T T 1680  
1681 T A T G T G A C G G A A G A A T A A A C T G T T G A T G G C A T 1712

GDF-3	KRRAAISVPKGFC--RNFCRRHQLFINF-QDLGWHKWVIAPKGFMANYPCHGE	PFPSMTTYLNS--
GDF-9	FNLSEYFKQFLFP--QNECELHDFRLSF-SQLKWDNWIIVAPHRYNPRY	CKGD
GDF-1	PRRDAEVLGGGP--GGA	RRARRLYVSF-REVGWHRWVIAPRGFLANY
Vg-1	RRKRSYSKLPFTA--SNI	CKRRHLYVEF-KDVGWQNWVIAPQGYMANYPYGE
Vgr-1	RVSSASDYNSEL--KTA	CKRKHLYVSF-QDLGWQDWIIAPKGYAANYDGE
OP-1	RMANVAENSSDQ--RQA	CKKHLYVSF-RDLGWQDWIIAPEGYAAYYEGE
BMP-5	RMSSVGDYNTSEQ--KQA	CKKHLYVSF-RDLGWQDWIIAPEGYAAYYDGE
60A	SPNNVPLLEPMES--TRS	COMQTLYIDF-KDLGWHDWIIAPEGYGAFYSGE
BMP-2	EKRQAKHKQRKRL--KSS	CKRHPLYVDF-SDVGWNDWIVAPPGYHAFYCHGE
BMP-4	RSPKHHSQRARKK--NKN	CKRRHSLYVDF-SDVGWNDWIVAPPGYQAFYCHGD
DPP	KRHARRPTRRKNH--DDT	CKRRHSLYVDF-SDVGWDDWIVAPLGYDAYYCHGK
BMP-3	QTLKKARRKQWIE--PRN	CKARRYLKVDF-ADIGWSEWIIISPKSFDAYYSGA
MIS	PGRQRSAGATAA--DGP	CKALRELSVDL---RAERSVLIPEITYQANNQGV
Inhibin $\alpha$	LRLLRPPEEPAA--HAN	CKHRVALNISF-QELGWERWIVYPPSFIFHYCHGC
Inhibin $\beta A$	RRRRRGLECDGKV--NI	CKKQFFVSF-KDIGWNDWIIAPSGYHANYEGE
Inhibin $\beta B$	RIRKRGLECDGRT--NI	CKCRQQFFIDF-RLIGWNDWIIAPTGYGNYEGS
TGF- $\beta 1$	RRALDTNYCFSST--EKN	CKCVRQLYIDFRKDLGWK-WIHEPKGYHANFELGF
TGF- $\beta 2$	KRALDAAYCFRNV--QDN	CKLRPLYIDFKRDLGWK-WIHEPKGYNANFAGA
TGF- $\beta 3$	KRALDTNYCFRNL--EEN	CKCVRPLYIDFRQDLGWK-WVHEPKGYANFCSGP
TGF- $\beta 4$	RRDLTDYCFGPGTDEKN	CKCVRPLYIDFRKDLQWK-WIHEPKGYMANFMGF
TGF- $\beta 5$	KRGVGQEYCFGNN--GPN	CKVKPLYINFRKDLGWK-WIHEPKGYEANYCLGN

FIG.3a

GDF-3 --SNYAFMQALMHM--ADPKVPKAVCV--PTKLSPISMLYQ--DSDKNVILRHYEDMVVDECCG  
 GDF-9 --PVHTMVQNIIE--KLDPSVPRPSCV--PGKYSPLSVLTI--EPDGSIAKEYEDMIATRIICOR  
 GDF-1 PALNHAIVLRALMHA--AAPGAADLPCCV--PARLSPISVLFF--DNSDNVVLQYEDMVVDECCOR  
 Vg-1 --SNHAILQTLVHS--IEPEDIPLPCCV--PTKMSPISMLFY--DNNDNVVLRHYENMAVDECCOR  
 Vgr-1 --TNHAIIVQTLVHL--MNPEYVPKPCCA--PTKLNAISVLVY--DDNSNVILKKYRNMVVRACCCH  
 OP-1 --TNHAIIVQTLVHF--INPETVPKPCCA--PTQLNAISVLVY--DDSSNVILKKYRNMVVRACCCH  
 BMP-5 --TNHAIIVQTLVHL--MFPDHVPKPCCA--PTKLNAISVLVY--DDSSNVILKKYRNMVVRSACCH  
 60A --TNHAIIVQTLVHL--LEPKKVPKPCCA--PTRLGALPVLVY--LNDENVNLKKYRNMIIVKSCCH  
 BMP-2 --TNHAIIVQTLVNS--VNSKIIPKACCV--PTELSAISMLYL--DENEKVVLKNYQDMVVEGCCOR  
 BMP-4 --TNHAIIVQTLVNS--VNSSIIPKACCV--PTELSAISMLYL--DEYDKVVLKNYQEMVVEGCCOR  
 DPP --TNHAVVQTLVNN--MNPCKVPKACCV--PTQLDSVAMLVY--NDQSTVVLKNYQEMTVVVGCCOR  
 BMP-3 --SNHATIOQIVRA--VGVVPGIPEPCV--PEKMSSLSILFF--DENKNVVLKVYPNMTVESCACOR  
 MIS --GNHVLLLLKMQA--RGAALARPCCV--PTAYAGKLLISLSEER--ISAHHVPNMVATECCOR  
 Inhibin  $\alpha$  --PGAPPTPAQPS--LLPGAQPCCAALPGTMRPLHVRRTSDGGYSFKYETVPNLLTQHACAI  
 Inhibin  $\beta$ A --SFHSTVINHYRMGRGHPFANLKS--PTKLRPMSMLY--DDGQNI IKKDIQNMIVEECES  
 Inhibin  $\beta$ B --SFHTAVNQYRMGRGLNPGT--VNSCCI--PTKLSTMSMLY--DDEYNIVKRDVPNMIVEECOA  
 TGF- $\beta$ 1 --TQYSKVLALYNQ--HNPGASAPCCV--PQALEPLPIVY--VGRKPKV--EQLSNMIVRSCKES  
 TGF- $\beta$ 2 --TQHSRVLSLYNT--INPEASAPCCV--SQDLEPLTILY--IGKTPKI--EQLSNMIVKSCCKES  
 TGF- $\beta$ 3 --TTHSTVLGLYNT--LNPEASAPCCV--PQDLEPLTILY--VGRTPKV--EQLSNMIVKSCCKES  
 TGF- $\beta$ 4 --TQYTKVLALYNQ--HNPGASAPCCV--PQTLDPPLIYY--VGRNVVR--EQLSNMIVVRACKES  
 TGF- $\beta$ 5 --TQYSKVLSLYNQ--NNPGASISPCCV--PDVLEPLPIYY--VGR TAKV--EQLSNMIVVRSCKES

FIG.3b

	TGF- $\beta$ 5	36	36	25	32	34	37	39	37	36	36	36	35	33	35
	TGF- $\beta$ 4	33	33	22	25	34	37	39	39	37	36	36	33	32	33
	TGF- $\beta$ 3	32	32	25	33	37	37	39	38	38	36	40	36	35	35
	TGF- $\beta$ 2	31	31	25	32	36	36	37	38	35	35	39	34	33	35
	TGF- $\beta$ 1	36	36	23	33	34	34	35	34	34	34	38	35	34	35
	INHIBIN $\beta$ B	41	41	31	35	37	41	41	42	37	37	39	42	42	42
	INHIBIN $\beta$ A	42	42	30	37	44	44	44	43	43	43	36	42	41	39
	INHIBIN $\alpha$	25	25	27	23	22	25	24	24	24	24	24	22	22	19
	MIS	22	22	21	34	30	24	27	27	24	25	25	27	27	25
	BMP-3	42	42	29	42	49	44	42	42	43	41	41	48	47	43
	DPP	47	47	32	41	48	59	58	58	57	54	54	74	75	100
	BMP-4	50	50	34	43	56	60	60	60	61	59	57	92	100	-
	BMP-2	53	53	33	42	58	61	60	61	61	57	57	100	-	-
	60A	47	47	30	41	51	71	69	74	74	100	100	-	-	-
	BMP-5	50	50	31	46	56	91	88	100	100	-	-	-	-	-
	OP-1	50	50	30	47	57	87	100	-	-	-	-	-	-	-
	Vgr-1	53	53	31	46	58	100	-	-	-	-	-	-	-	-
	Vg-1	57	57	30	57	100	-	-	-	-	-	-	-	-	-
	GDF-1	50	50	27	100	-	-	-	-	-	-	-	-	-	-
	GDF-9	33	33	100	-	-	-	-	-	-	-	-	-	-	-
	GDF-3	100	100	-	-	-	-	-	-	-	-	-	-	-	-
GDF-3		100	100	-	-	-	-	-	-	-	-	-	-	-	-
GDF-9		-	100	27	30	57	100	-	-	-	-	-	-	-	-
GDF-1		-	-	100	57	100	-	-	-	-	-	-	-	-	-
Vg-1		-	-	-	100	58	100	-	-	-	-	-	-	-	-
Vgr-1		-	-	-	-	100	87	91	71	-	-	-	-	-	-
OP-1		-	-	-	-	-	100	88	69	-	-	-	-	-	-
BMP-5		-	-	-	-	-	-	100	74	-	-	-	-	-	-
60A		-	-	-	-	-	-	-	100	-	-	-	-	-	-
BMP-2		-	-	-	-	-	-	-	-	100	92	74	-	-	-
BMP-4		-	-	-	-	-	-	-	-	-	100	75	-	-	-
DPP		-	-	-	-	-	-	-	-	-	-	100	-	-	-

FIG.4a

TGF- $\beta$ 5	30	26	24	36	28	82	70	73	79	100
TGF- $\beta$ 4	27	29	24	33	30	86	68	74	100	-
TGF- $\beta$ 3	32	25	24	36	37	78	82	100	-	-
TGF- $\beta$ 2	32	23	22	37	34	74	100	-	-	-
TGF- $\beta$ 1	32	28	23	41	35	100	-	-	-	-
INHIBIN $\beta$ B	37	25	25	63	100	-	-	-	-	-
INHIBIN $\beta$ A	36	24	26	100	-	-	-	-	-	-
INHIBIN $\alpha$	29	18	100	-	-	-	-	-	-	-
MIS	30	100	-	-	-	-	-	-	-	-
BMP-3	100	-	-	-	-	-	-	-	-	-
DPP	-	-	-	-	-	-	-	-	-	-
BMP-4	-	-	-	-	-	-	-	-	-	-
BMP-2	-	-	-	-	-	-	-	-	-	-
60A	-	-	-	-	-	-	-	-	-	-
BMP-5	-	-	-	-	-	-	-	-	-	-
OP-1	-	-	-	-	-	-	-	-	-	-
Vgr-1	-	-	-	-	-	-	-	-	-	-
Vg-1	-	-	-	-	-	-	-	-	-	-
GDF-1	-	-	-	-	-	-	-	-	-	-
GDF-9	-	-	-	-	-	-	-	-	-	-
GDF-3	-	-	-	-	-	-	-	-	-	-
BMP-3										
MIS										
INHIBIN $\alpha$										
INHIBIN $\beta$ A										
INHIBIN $\beta$ B										
TGF- $\beta$ 1										
TGF- $\beta$ 2										
TGF- $\beta$ 3										
TGF- $\beta$ 4										
TGF- $\beta$ 5										

FIG.4b

9/15

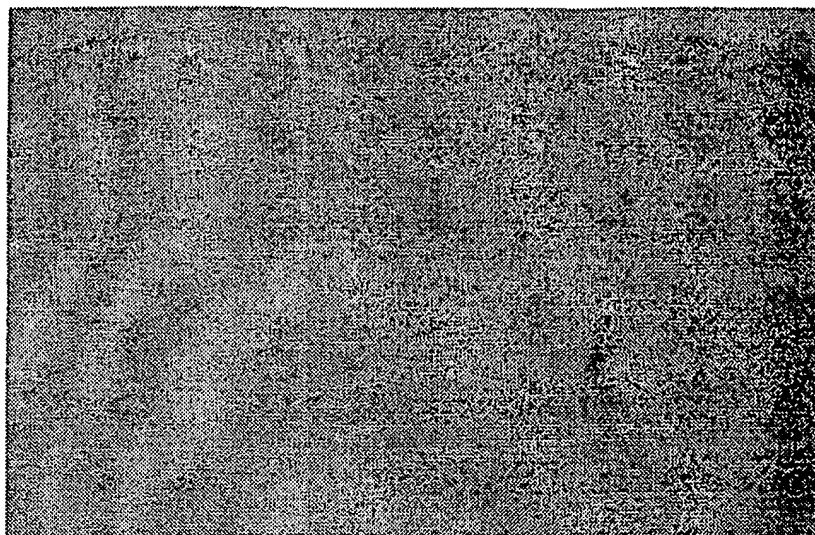


FIG. 5c

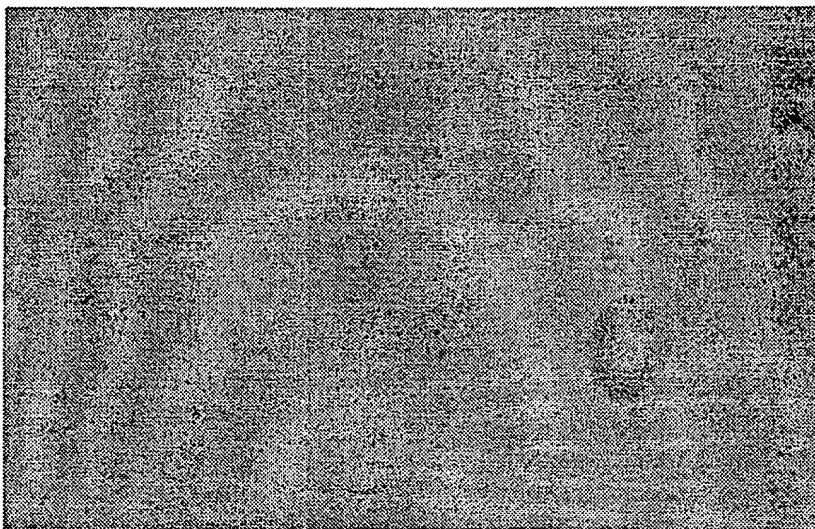


FIG. 5b

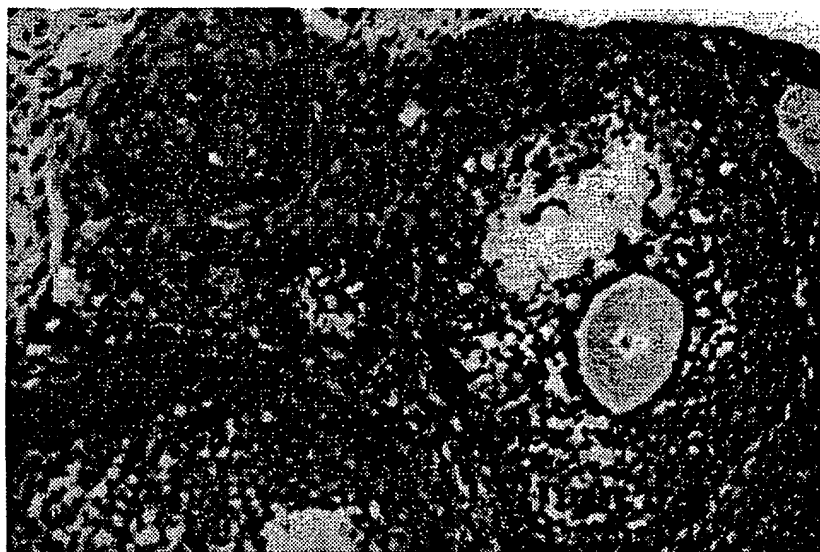


FIG. 5a

1 MALPSNFFLLGVCCFAWLCFLSSLSSQASTEESQSGASENVESEADPWSLL 50  
 || | ||| ||||| || ||| | | ||| |||  
 1 MARPNKFLWFWCCFAWLCFPISLGSQASGGEAQIAASAELESGAMPWSLL 50  
  
 51 LPVDGTDRSGLLPPLFKVLSDRRGETPKLQPDSTRALYYMKKLYKTYATKE 100  
 | || ||| ||||| || | ||||| ||||| |||||  
 51 QHIDERDRAGLLPALFKVLSVGRGGSPRLQPDSTRALHYMKKLYKTYATKE 100  
  
 101 GVPKPSRSHLYNTVRLFSPCAQQEQAPSNOVTGPLPMVDLLFNLDRTAM 150  
 | || ||||| ||| || ||| || | ||||| |  
 101 GIPKSNRSHLYNTVRLFTPCTRHKQAPGDQVTGILPSVELLFNLDRTTV 150  
  
 151 EHLLKSVLLYTLNNSASSSSTVTMCDLVVKEAMSSGRAPPRAPYSFTL. 199  
 ||||| ||| | | | | | | | | | |||||  
 151 EHLLKSVLLYNINNSVSFSSAVKVCNLMIKEPKSSSRTLGRAPYSFTFN 200  
  
 200 .....KKHRWIEIDVTSLLQPLVTSSERSIHLSVNFTCTKDQV....PE 239  
 ||| || ||||| || | ||| | ||| |||  
 201 SQFEFGKKHKWIIQIDVTSLLQPLVASNKRSIHMSINFCTMKDQLEHPSAQ 250  
  
 240 DGVFSMPLSVPPSLILYLNDTSTQAYHSWQSLQSTWRPLQHPGQA.GVAA 288  
 | | | | | ||||| ||||| || | | | | |  
 251 NGLFNMTL.VSPSLILYLNDTSAQAYHSWYSLHYKRRPSQGPDPERSLSA 299  
  
 289 RPVKEEATEVERSP..RRRRGQKAIRSEAKGPLLTASFNLSEYFKQFLFP 336  
 || ||| | || | ||| || | || | ||||| ||| |  
 300 YPVGEEAAEDGRSSHRRRGQETVSSELKKPLGPASFNLSEYFRQFLLP 349  
  
 337 QNEELHDFRLSFSQLKWDNWIVAPHRYNPRYKGDPRAVRHRYGSPVH 386  
 ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
 350 QNEELHDFRLSFSQLKWDNWIVAPHRYNPRYKGDPRAVGHRYGSPVH 399  
  
 387 TMVQNIIEKLDPSVPRPSVPGKYSPLSVLTIEPDGSIAYKEYEDMIAT 436  
 ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
 400 TMVQNIIEKLDSSVPRPSVPAKYSPLSVLTIEPDGSIAYKEYEDMIAT 449  
  
 437 RIIIR 441  
 ||||  
 450 KIIIR 454  
 ||||



64%

FIG. 6

90%

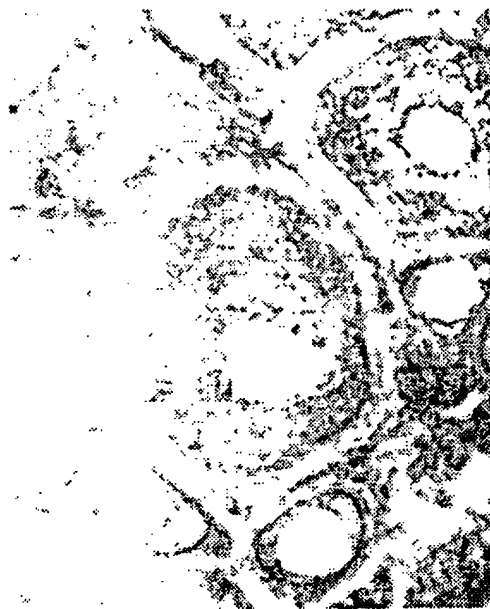
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11/15

**FIG. 7a**



**FIG. 7b**

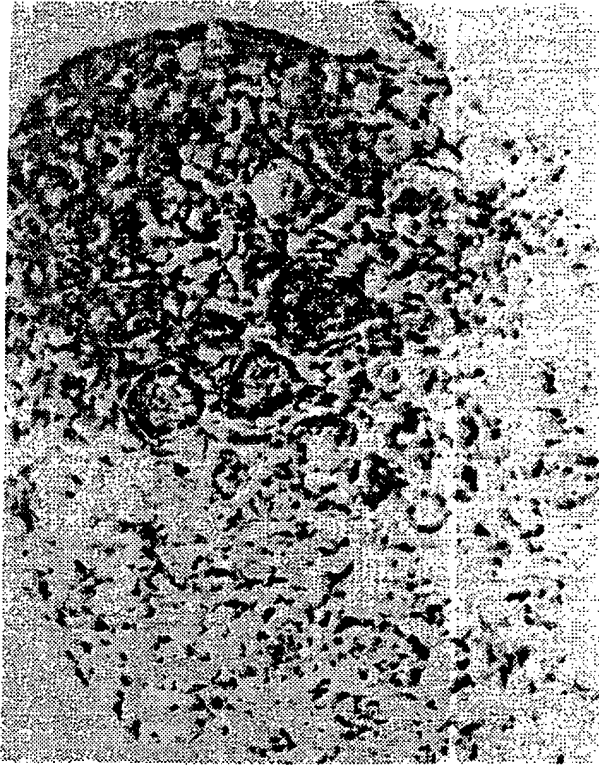


**FIG. 7c**

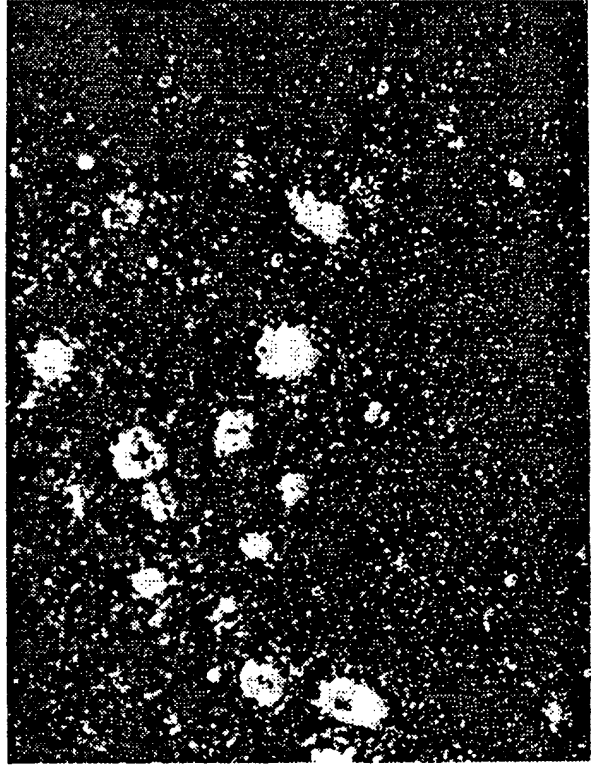


**FIG. 7d**

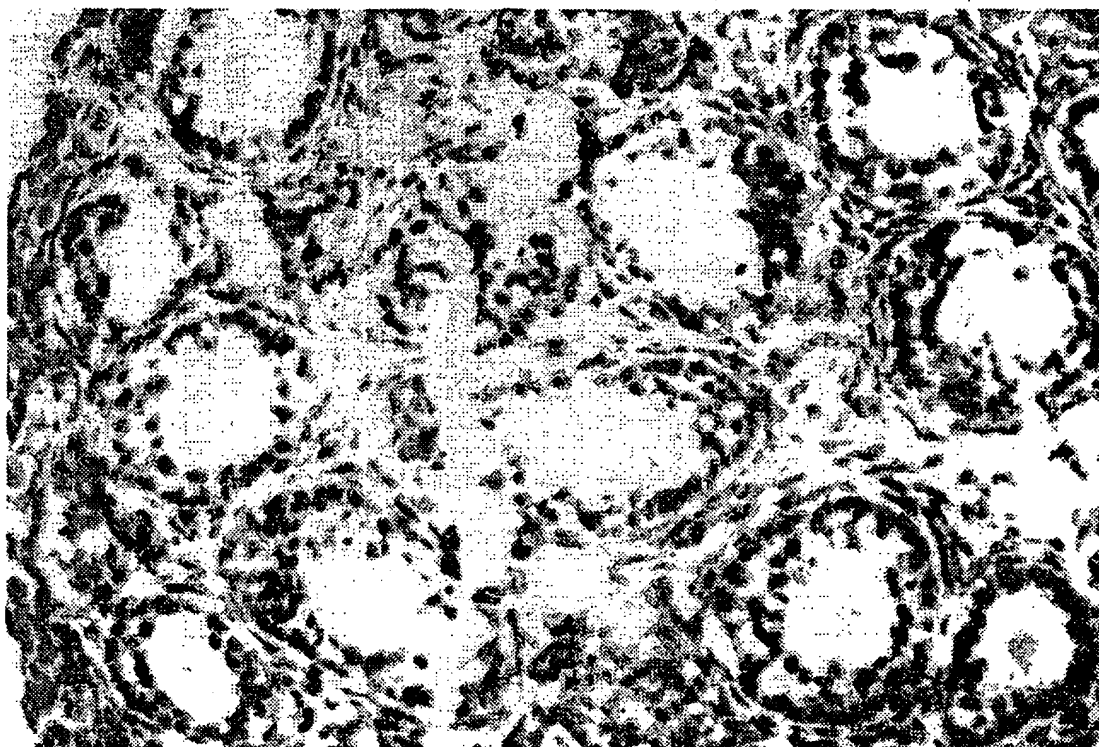
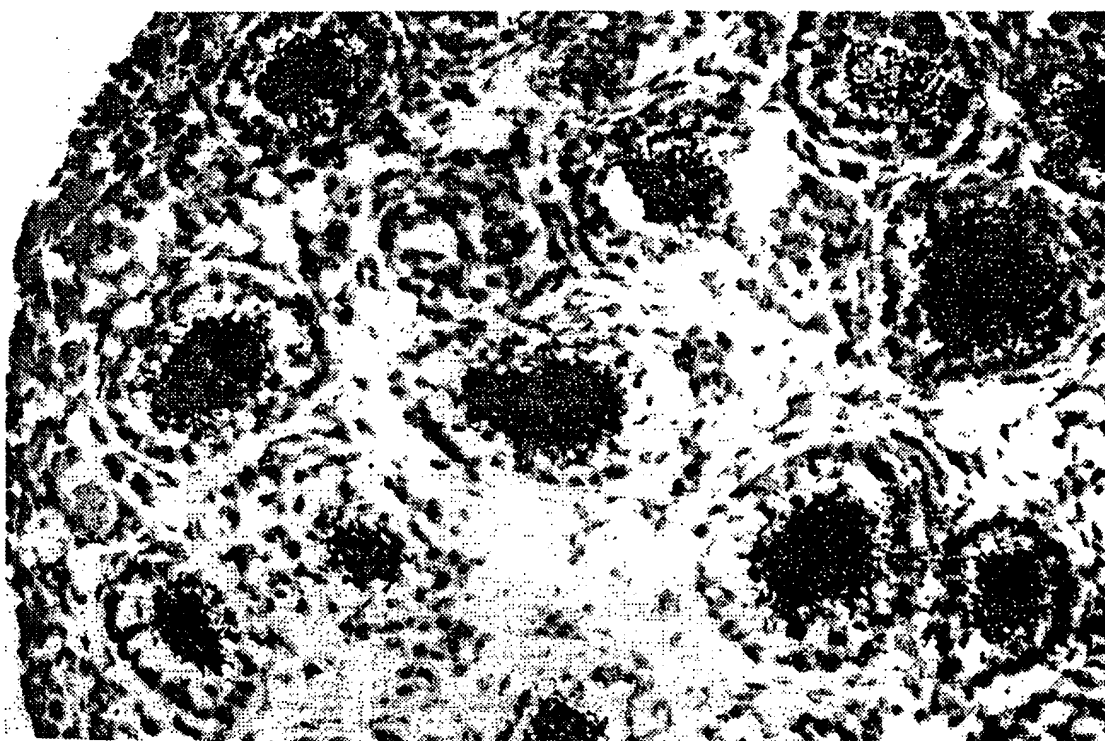
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**FIG. 8a**



**FIG. 8b**

**FIG. 9b****FIG. 9a**

14/15

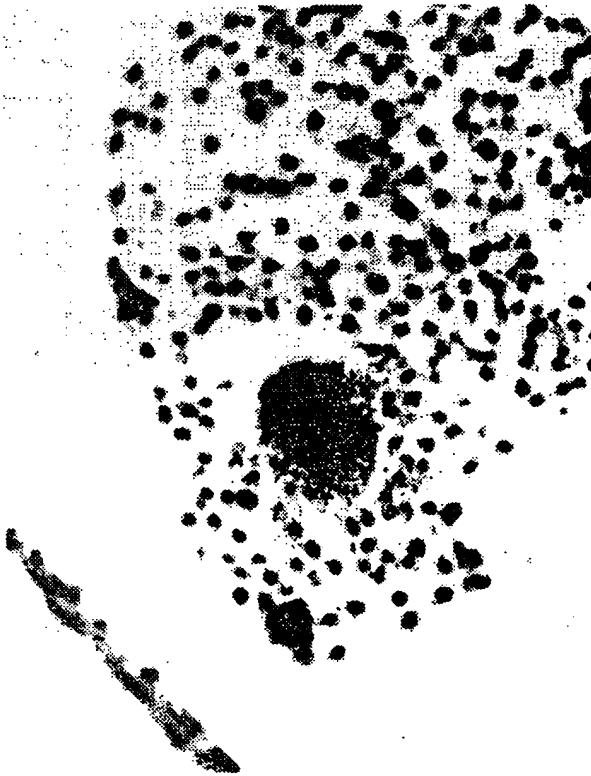
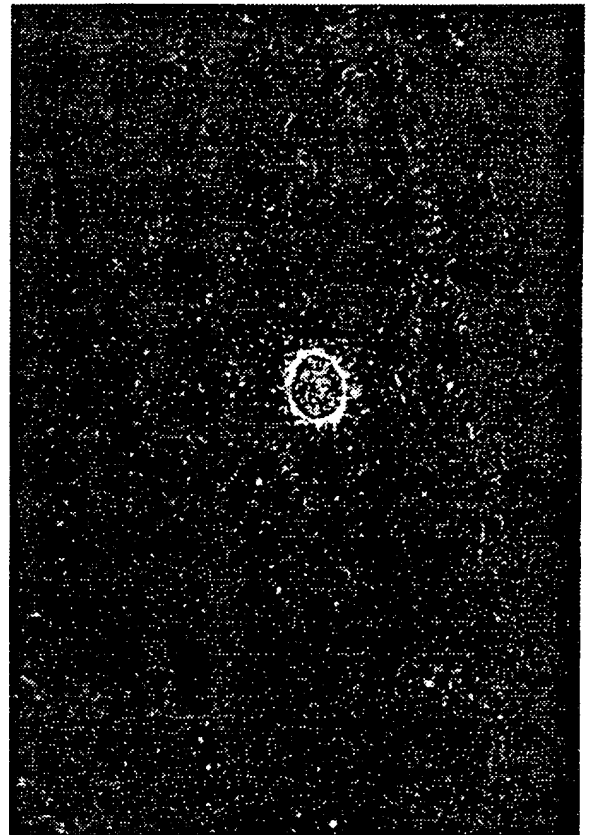


FIG. 10a



FIG. 10b

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**FIG. 11a****FIG. 11b**

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :C07K 13/00, 15/28; A61K 37/36; C12N 15/11, 15/18

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5, 23.4; 435/320.1, 69.1, 69.4, 91.1, 91.4, 252.3, 252.33; 530/350, 399, 388.23

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog, APS search terms: growth differentiation factor-9

Sequence search: GenBank, GeneSeq, PIR, SwissPro

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOLECULAR ENDOCRINOLOGY, Volume 4, issued 1990, Lee, "Identification of a Novel Member (GDF-1) of the Transforming Growth Factor-beta Superfamily," pages 1034-1039.	1-39
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, Volume 88, issued May 1991, Lee, "Expression of growth/differentiation factor I in the nervous system: Conservation of a bicistronic structure", pages 4250-4254, see entire document.	1-39

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

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Date of the actual completion of the international search

28 MARCH 1994

Date of mailing of the international search report

25 APR 1994

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Facsimile No. (703) 305-3230

Authorized officer

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## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	THE JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 268, Number 5, issued 15 February 1993, McPherron et al., "GDF-3 and GDF-9:Two New Members of the Transforming Growth Factor-beta Superfamily Containing a Novel Pattern of Cysteines", pages 3444-3449, see figure 2.	1-39

**A. CLASSIFICATION OF SUBJECT MATTER:**

US CL :

536/23.5, 23.4; 435/320.1, 69.1, 69.4, 91.1, 91.4, 252.3, 252.33; 530/350, 399, 388.23

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